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TITLE: UCLA High Speed, High Volume Laboratory Network for Infectious Diseases

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14. ABSTRACT
Background: To be able to respond proactively to infectious disease threats at the population level, laboratories are needed that capable of analyzing tens of thousands of biological samples per day and handling the resulting large sets of data. The High Speed, High Volume Laboratory at UCLA will vastly increase the rate at which infectious agents are submitted, tested, and analyzed. This enhanced capacity will improve our nation's ability to respond quickly to a bio-emergency, such as a bio-terrorist attack or an influenza pandemic. **Objectives/Hypothesis:** This project aims to develop a new kind of high-throughput laboratory and informatic capability. It will provide a platform (high-speed high-volume processing, accessioning, archiving, screening, genotyping, culturing, phenotyping) for data-driven science and discovery. This platform is first being developed for influenza and can be expanded to include other pathogens as well. Integrated bioinformatics will associate surveillance information from the field with laboratory data and enable data-driven science and discovery against emerging and engineered biothreats. **Specific Aims:** With FY06 (Initial Year), FY07 (OY1) and FY08 (OY2) Congressional appropriations, high-throughput bioagent *Automated Genotyping System* has been implemented first and are currently in the process of purchasing an initial unit of the *Automated Archiving System*. In addition, 100% specifications for both the *Automated Culturing System* and *Automated Screening System* have been produced; RFPs have been released by UCLA Purchasing in February 2010. The *Automated Screening System* will be purchased with OY2 funds. With FY09 (OY3) Congressional appropriations, the *Automated Culturing System* will be purchased. In addition, OY3 funds will be used to complete specifications for the *Automated Phenotyping System*. If FY10 (OY4) Congressional appropriations are approved (or if other funds become available), these funds will be used to purchase the *Automated Phenotyping System* and two additional units of the *Automated Archiving System*. The automated systems will be housed in laboratory space at UCLA that has been upgraded to BSL3-enhanced (BSL3e) containment (to be completed in OY2) that enables the flow of numerous samples containing Highly Pathologic Avian Influenza and other Select Agents (dual-use). **Study Design:** Because of current public health and national security threats, influenza surveillance and analysis will be the initial focus. In the upcoming project period, the project will be expanded to include other biothreat agents, bacterial and/or viral. **Relevance:** The combination of high-throughput and automated systems will enable processing of tens of thousands of samples and provide critical laboratory capacity. Its overall design and implementation will facilitate expansion to multiple networked sites. As called for in Homeland Security Presidential Directive 21, there are important needs for near-real time biosurveillance in the event of a catastrophic health event (whether civilian or military). The high-throughput capability will be a critical addition to the DoD's chemical and biological defense and infectious disease programs, including the Transformational Medical Technologies Initiative (TMTI).

15. SUBJECT TERMS

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INTRODUCTION

Background: To be able to respond proactively to infectious disease threats at the population level, laboratories are needed that capable of analyzing tens of thousands of biological samples per day and handling the resulting large sets of data. The High Speed, High Volume Laboratory at UCLA will vastly increase the rate at which infectious agents are submitted, tested, and analyzed. This enhanced capacity will improve our nation's ability to respond quickly to a bio-emergency, such as a bio-terrorist attack or an influenza pandemic. **Objectives/Hypothesis:** This project aims to develop a new kind of high-throughput laboratory and informatic capability. It will provide a platform (high-speed high-volume processing, accessioning, archiving, screening, genotyping, culturing, phenotyping) for data-driven science and discovery. This platform is first being developed for influenza and can be expanded to include other pathogens as well. Integrated bioinformatics will associate surveillance information from the field with laboratory data and enable data-driven science and discovery against emerging and engineered biothreats. **Specific Aims:** With FY06 (Initial Year), FY07 (OY1) and FY08 (OY2) Congressional appropriations, the high-throughput bioagent *Automated Genotyping System* has been implemented first, and we are currently in the process of purchasing an initial unit of the *Automated Archiving System*. In addition, 100% specifications for both the *Automated Culturing System* and *Automated Screening System* have been produced; RFPs have been released by UCLA Purchasing in February 2010. The *Automated Screening System* will be purchased with OY2 funds. With FY09 (OY3) Congressional appropriations, the *Automated Culturing System* will be purchased. In addition, OY3 funds will be used to complete specifications for the *Automated Phenotyping System*. If FY10 (OY4) Congressional appropriations are approved (or if other funds become available), these funds will be used to purchase the *Automated Phenotyping System* and two additional units of the *Automated Archiving System*. The automated systems will be housed in laboratory space at UCLA that has been upgraded to BSL3-enhanced (BSL3e) containment (to be completed in OY2) that enables the flow of numerous samples containing Highly Pathologic Avian Influenza and other Select Agents (dual-use). **Study Design:** Because of current public health and national security threats, influenza surveillance and analysis will be the initial focus. In the upcoming project period, the project will be expanded to include other biothreat agents (bacterial and/or viral). **Relevance:** The combination of high-throughput and automated systems will enable processing of tens of thousands of samples and provide critical laboratory capacity. Its overall design and implementation will facilitate expansion to multiple networked sites. As called for in Homeland Security Presidential Directive 21, there are important needs for near-real time biosurveillance in the event of a catastrophic health event (whether civilian or military). The high-throughput capability will be a critical addition to the DoD's chemical and biological defense and infectious disease programs, including the Transformational Medical Technologies Initiative (TMTI).

Keywords. Influenza; Real-Time Surveillance; High-Throughput Automation; Actionable Information; Pathomics; Surge Capacity; Transformational Medical Technologies Initiative

BODY

The final addendum report (revised) is being submitted at the request of Dr. Michael A. Smith, who disapproved the final addendum report (original) submitted on 23 July, 2009. The final addendum report (original) served an update to the annual report on the "UCLA High Speed, High Volume Laboratory Network for Infectious Diseases: Phase I" that was submitted on April 10, 2008 and addendum report that was submitted on February 2, 2009. The work covered was accomplished through two serial No Cost Extensions (NCEs) for the CLIN0001 component of funding and is summarized in terms of our approved Statement of Work (SOW). The two NCEs were used to procure materials and supplies (M&S) for a large-scale (600 samples) whole influenza B genome sequencing run at LANL with the new automated genotyping system from Agilent/Velocity 11 that was delivered in June 2009.

We recently conducted an extensive mid-course assessment of this project. In our FY06 (Initial Year) Statement of Work, we described proposed **Tasks 1-5** (**Phase I)**. Additional work proposed in the Statement of Work for FY07/08 (**Phase II)** is described below as **Tasks 6-9**. In the **BODY** section, we summarize each task of the original proposed scope of work, and summarize the results of the mid-course assessment and our progress to date.

Immediately below are bulleted lists of the proposed work to be funded by FY09 and FY10 (Option Year 3 and Option Year 4) for each task. Following this, **Figures 1** and **2** provide high level overviews of the laboratory work flows and **Table 1** provides a summary of the mid-course assessment. Next, we summarize all nine tasks according to: (A) the original proposed scope of work; (B) the results of the mid course assessment and our progress to date; and (C) the work that currently is being performed with available funding.

Task 1: LANL & UCLA. Assemble two automated systems that perform high-throughput tests.

Proposed Work on Task 1 in OY3:

- Assemble and validate *Automated Screening System* at LANL.
- Develop web-services for both the *Automated Genotyping* and *Automated Screening Systems*.

Task 2: LANL & UCLA. Build an operating system that runs and manages networking of multiple high-throughput laboratories.

Proposed Work on Task 2 in OY3:

- Complete the integration of the *Automated Accessioning, Genotyping,* and *Archiving Systems*.
- Lay the foundation for integration of the *Automated Culturing* and *Screening Systems*.
- Identify and implement further applications of the web portal.
- Perform further configuration of the LIMS and integrations with laboratory systems.

Proposed Work on Task 2 in OY4:

- Upgrade network and server systems.

Task 3: UCLA. Upgrade ~2,000 sq. ft. of floor space to BSL3e specifications to house high-throughput automated systems.

Proposed Work on Task 3 in OY3:

- Validate *Automated Genotyping, Accessioning,* and *Archiving Systems* under BSL3 conditions in upgraded facility at UCLA.
- Complete and submit Select Agent Registration Package to Centers for Disease Control and Prevention (CDC).
- Provide documentation of final laboratory commissioning to CDC and Department of Defense (DOD) to obtain clearance to perform testing using BSL3 agents.

Task 4: LANL & UCLA. Formulate quality management program and protocols, and establish team that operates the high-throughput laboratory.

Proposed Work on Task 4 in OY3:

- Finalize system and method development for *Automated Phenotyping System.*
- Perform and prepare lab-wide biosafety and biosecurity risk assessment.
- Prepare laboratory and agent specific biosafety and biosecurity manuals, and develop CNSI BSL3e incident response plan.
- Establish biosafety and biosecurity training curriculum, checklist, and schedule for BSL3e staff members.

Task 5: LANL & UCLA. Conduct large-scale, data-driven research on virulence, transmissibility and host range of influenza viruses.

Proposed Work on Task 5 in OY3:

- Continue large-scale, data-driven research on virulence, transmissibility and host range of influenza viruses.
- Continue extension of capabilities to other biothreat agents, including viruses and bacteria.

Task 6: UCLA & LANL. *Automated Archiving System*.

Proposed Work on Task 6 in OY3:

- Complete integration of the *Automated Archiving System.*

Proposed Work on Task 6 in OY4:

- Purchase two additional units for *Automated Archiving System.*

Task 7: LANL & UCLA. *Automated Culturing System*.

Proposed Work on Task 7 in OY3:

- Purchase *Automated Culturing System* and *Biocel* enclosure for system.
- Assemble and validate *Automated Culturing System* at LANL.

Task 8: LANL & UCLA. *Automated Phenotyping System*.

Proposed Work on Task 8 in OY3:

- Complete design and specifications for *Automated Phenotyping System*

Proposed Work on Task 8 in OY4:

- Purchase *Automated Phenotyping System* and *Biocel* enclosure for system.

Task 9: LANL & UCLA. Integration of *Automated Accessioning System*.

Proposed Work on Task 9 in OY3:

- Complete integration of the *Automated Accessioning System.*

Proposed Work on Task 9 in OY4:

- Purchase extended service contract for *Automated Accessioning System.*

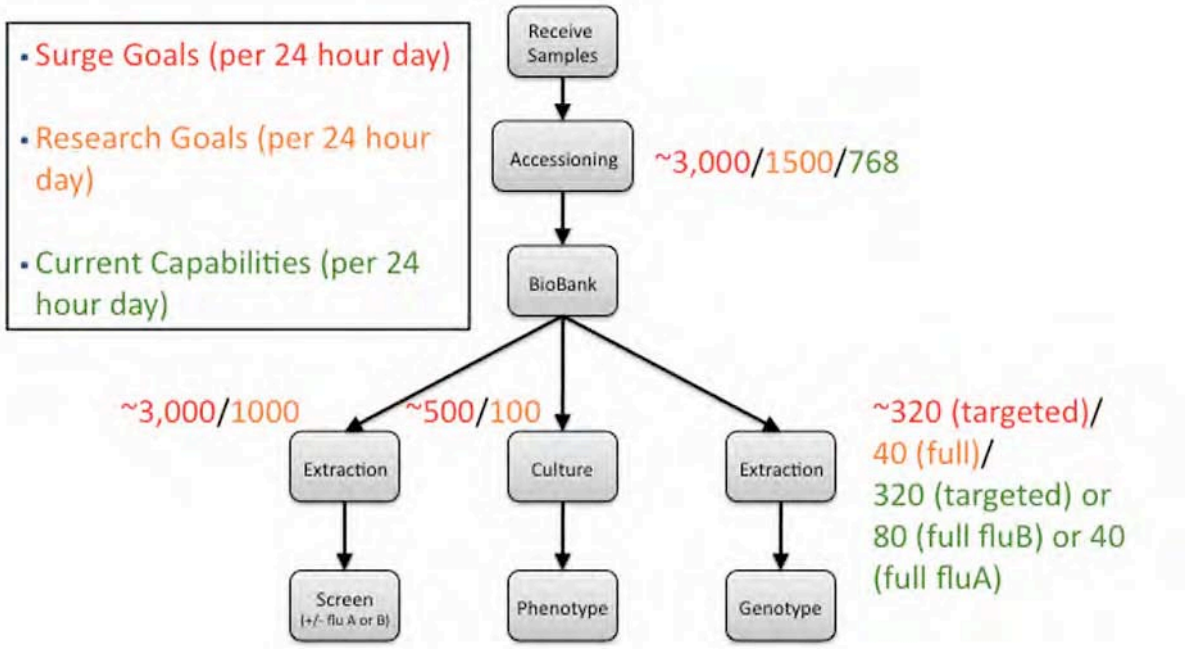

Figure 1. High level overview of the HTLN system architecture, showing designed capacity in terms of numbers of samples to be processed per day in pandemic or surge mode.

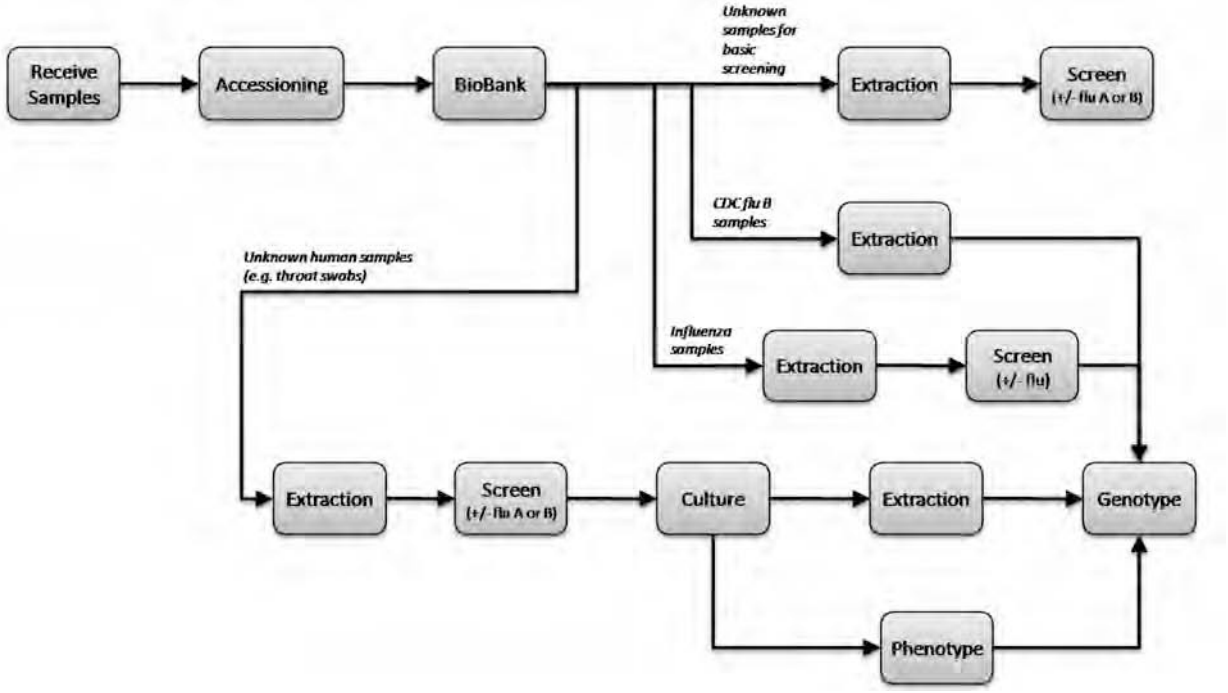

Figure 2. High level overview of logical flow of samples through the HTLN. The four different paths shown here for example depict four possible sample types

Table 1. Task Progression and Gap Analysis from Mid-Course Assessment

	PAST		CURRENT	FUTURE	
	March 2007-February 2008	March 2008-February 2009	March 2009-February 2010	March 2010-February 2011	March 2011-February 2012
TASKS	Year 1 FY06	OY1 FY07	OY2 FY08 (FUNDING GAPS SHADED IN GOLD)	OY3 FY09 (FUNDING GAPS SHADED IN GOLD)	OY4 FY10 (FUNDING GAPS SHADED IN GOLD)
Task 1: LANL & UCLA. Assemble two automated systems that perform high-throughput tests					
A. Automated Screening System	Initiate design	Continue design	Complete design and purchase;	Assemble and validate at LANL	*Move to upgraded facility at UCLA and install under BSL3; (additional resources needed)*
B. Automated Genotyping System	Design and purchase	Assemble and validate at LANL	Move to upgraded facility at UCLA and install under BSL2	Validate at UCLA under BSL3	*Operational (additional resources needed)*
Task 2: LANL & UCLA. Build an operating system that runs and manages networking of multiple high-throughput laboratories.	Initiate development; Purchase LIMS	Continue development	*Continue development (additional resources needed)*	*Continue development (additional resources needed)*	Purchase additional software licenses *Continue development (additional resources needed)*
Task 3: UCLA. Upgrade ~2,000 sq. ft. of floor space to BSL3e specifications for housing high-throughput automated systems.	Initiate upgrade	Continue upgrade	Complete upgrade; Obtain certifications for BSL2 work	Obtain certifications for BSL3 and Select Agent work	*Obtain CLIAA certification (additional resources needed)*
Task 4: LANL & UCLA. Formulate quality management program and protocols, and establish team that operates the high-throughput laboratory.	Initiate	Continue	Continue	*Continue (additional resources needed)*	*Continue (additional resources needed)*
Task 5: UCLA & LANL. Conduct large-scale, data-driven research on virulence, transmissibility and host range of influenza viruses.	Initiate	Continue	Continue	*Continue (additional resources needed)*	*Continue (additional resources needed)*
Task 6: UCLA & LANL. Automated Archiving System	Determine required specifications and identify potential vendors	Identify preferred vendor	Purchase first unit; Install in upgraded facility at UCLA under BSL2	Validate at UCLA under BSL3	Purchase two additional units; Install in upgraded facility at UCLA under BSL3
Task 7: LANL & UCLA. Automated Culturing System	Initiate design	Continue design	Complete design	Purchase; Assemble and validate at LANL	*Move to upgraded facility at UCLA and install under BSL3; (additional resources needed)*
Task 8: LANL & UCLA. Automated Phenotyping System			Initiate design	Complete design	Purchase *Assemble and validate at LANL; Move to upgraded facility at UCLA and install under BSL3; (additional resources needed)*
Task 9: LANL & UCLA. Integration of Accessioning System	*Design and purchase (performed with other funds)*	Assemble and validate at LANL	Move to upgraded facility at UCLA and install under BSL2	Validate under BSL3	*Operational (additional resources needed)*

Task 1: LANL & UCLA. Assemble two automated systems that perform high-throughput tests.

A. Summary of original proposed scope of work for Task 1. The original proposal (FY06 request) was to develop an *Automated Screening Syst*em to determine whether individual samples contain influenza viruses. The proposed *Automated Screening Syst*em would determine the type and subtype of samples so that the primers required for amplification cane be selected prior to sequencing. We proposed to develop an *Automated Genotyping System* to be used to sequence whole viral genomes and/or individual gene segments. The two systems were to be assembled to exact specifications by a chosen vendor (which at the time of the original proposal was anticipated to be Velocity11). An established team at LANL was designated responsibility for validating the systems, which subsequently would be installed in the upgraded BSL3 facility at UCLA.

B. Mid-course Assessment and Progress on Task 1. The *Automated Genotyping System* (see **Figures 3** and **4** below) was developed through close coordination between LANL and UCLA. Velocity 11 (later acquired by Agilent Technologies) was selected as the systems integrator for the *Automated Genotyping System* based on competitive proposals submitted by potential vendors in response to an openly-advertised Request for Proposals released in March 2008. The *Automated Genotyping System* was delivered and installed at LANL in May 2009, and is currently being tested at LANL to establish reliability and performance expectations. To validate the *Automated Genotyping System*, we are performing an initial large scale test run to sequence the genomes of 600 Influenza B samples obtained from the CDC (see Task 5 below).

The actual cost of the *Automated Genotyping System* ($1.76 M) was significantly more than originally anticipated ($780,000). Work performed at LANL and UCLA to optimize viral extraction protocols revealed that the majority of the influenza surveillance samples that are likely to come to the facility do not have a high enough viral load to obtain the genotype without prior amplification (*i.e.*, without culturing of virus extracted from the sample). As a result, the team decided that the *Automated Culturing System* should be made the next highest priority for development. Both of these factors resulted in a delay in the acquisition of the *Automated Screening System* from what we originally proposed. In January 2010, LANL and UCLA completed 100% design plans for the *Automated Screening System* and forwarded them to UCLA Purchasing. The Request for Proposals (RFPs) for the *Automated Culturing System* (see Task 7 below) and *Automated Screening System* were released and advertised by UCLA Purchasing in February
2010.

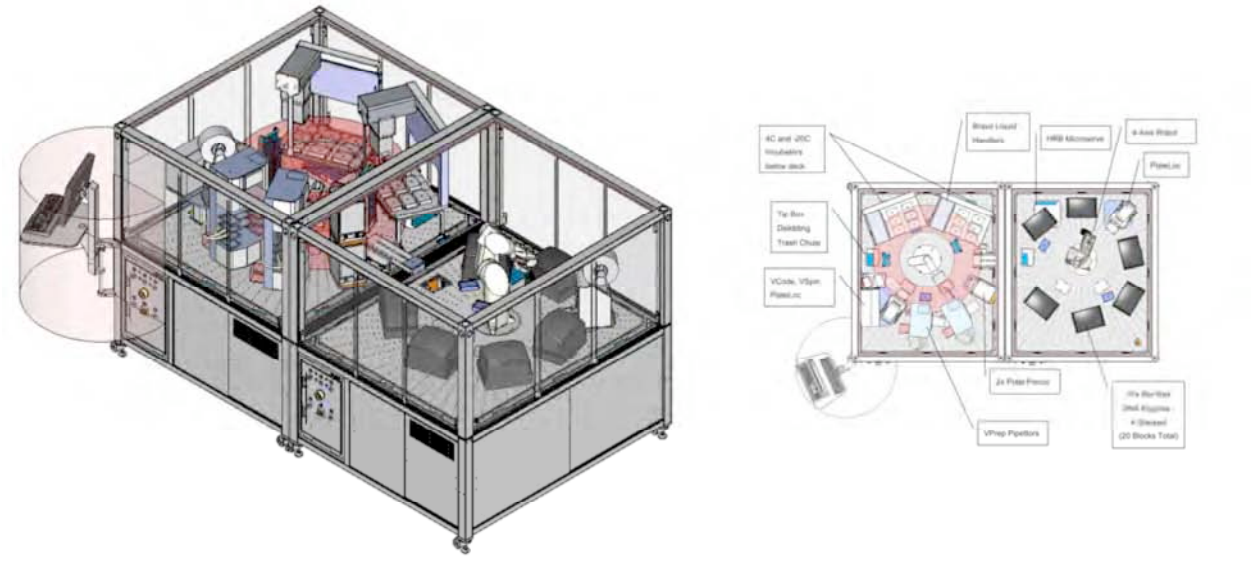

Figure 3. Computerized-generated images of the automated genotyping system.

Figure 4. Two views of the automated genotyping system, which is undergoing validation and testing at LANL.

C. Ongoing Work in OY2 on Task 1. When we complete the trial run on the 600 Influenza B samples from the CDC, analyze the resulting data and determine those data to be of sufficient quality, the *Automated Genotyping System* will be transferred to UCLA (anticipated April 2010). The *Automated Genotyping System* will arrive while the facility is being operated under BSL2 conditions (a necessary prerequisite to being certified to handle BSL3 and Select Agents) and hence will be installed at UCLA under BSL2 conditions. (Note: To Date, 378 out of 600 samples that can yield whole influenza genomes have been processed once by the *Automated Genotyping System*, see Task 5 below.) In addition, funds from OY2 will be used to purchase the *Automated Screening System*, which will be delivered to LANL for initial installation and validation.

Task 2: LANL. Build an operating system that runs and manages networking of multiple high-throughput laboratories.

A. Summary of original proposed scope of work for Task 2. The original proposal for FY06 (Initial Year) was to develop an operating system that allows access by way of the Internet, to enable flexible and programmable testing procedures, schedule and control numerous tests, and deposit results into an Internet-enabled database for analysis, geospatial mapping and real-time display. An established team at LANL was designated to perform this task.

B. Mid-course Assessment and Progress on Task 2. We hired an Information Technology (IT) Manager, Alexander Roth, at UCLA to collaborate with the established team at LANL. With FY06 (Initial Year) DOD support, we began work on the operating system, whose architecture is based on three United States Patents (Layne and Beugelsdijk 1998, 1999 and 1999). The operating system uses the Laboratory Equipment Control Interface Specification (American Society for Testing and Materials 1999) coupled with various commercial hardware and software packages.

IT Architecture. With FY07 (Option Year 1) support, we started enhancing the basic operating system and implementing an n-Tier IT architecture that enables computing platform-independent computing, vendor-neutral data storage and retrieval, plug-and-play compatibility and scalability. To achieve these features, the operating system will be logically partitioned into five layers (Physical, Data, Business Logic, Presentation, and Application). We will use open IT standards, such as web services for interoperability across heterogeneous computing platforms, eXtensible Markup Language (XML) for data exchange, and Open Database Connectivity (ODBC). **Table 2** outlines the purpose and function of these five layers.

Table 2. n-Tier IT Architecture

Layers	Description
Application	The Application layer hosts internal and external, business-to-business and/or user applications.
Data	The Data layer manages all data generated by or submitted to the laboratory. Databases, flat files and backup tapes are examples of items residing at this level.
Business Logic	The Business Logic layer defines how the laboratory operates internally and how to conduct business with it (e.g., how to submit samples, process samples, manage report and conduct Enterprise Resource Planning (ERP).
Presentation	The Presentation layer defines how information is exchanged and presented to applications from the Application Layer (e.g., via web forms, ASP.NET server pages and XML/SOAP).
Physical	The Physical layer collectively refers to all laboratory facilities, automated (and non-automated) laboratory systems and functional subsystems.

In FY06 (Initial Year) and continuing in FY07 (Option Year 1), we have undertaken product research and analysis on different IT components, which are needed to operate the high-throughput laboratory network and host databases with surveillance metadata, genotypic data, phenotypic data and internal lab data. To support this effort, we purchased two identical servers suitable to act as data servers and two additional servers suitable to act as webservers. Webserver and data server were installed at UCLA and LANL. We are in the process of replicating the systems between LANL and UCLA in order to create a redundant and fault tolerant failover system to ensure reliable system operation and availability. These physically separate sites protect data from loss in the event of system failure or natural disaster. We worked with engineers at UCLA's central Data Center to identify and reserve rack space for the server systems with all of the necessary power and network connections. Based on this work, the web and data servers were placed into the UCLA Data Center, which provides 24/7/365 operational support. Similar efforts were undertaken at LANL where the identical server system was placed and connected to the network.

IT Specifications for and Integration of Automated Systems. IT specifications have been completed for the *Software Control Interface for Laboratory Systems and Automated Genotyping System,* are being finalized for the *Automated Culturing System* and are in progress for the *Automated Screening* and *Archiving Systems*. We have made considerable progress on the development of: (1) web service-based instrument-specific control interface for *Automated Genotyping System*, (2) a mechanism to ensure server high availability and fault-tolerance, (3) specifications for the web-OS architecture, and (4) specifications for the IT laboratory security concept. Moreover, we have initiated work to address regulations for Select Agent data. We purchased a LIMS system from StarLIMS using FY06 (Initial Year) funds which has been installed and setup on the local servers at UCLA (and mirrored at LANL). Integration of the *Automated Accessioning, Genotyping Systems* with the LIMS and overall IT architecture has been initiated utilizing web services. The concept for the web portal has been created and we continue to work on its core functionality. We have also commenced developing configurations and integrations with laboratory systems.

The mid-course assessment underscored the applicability of integrated IT architecture being developed as part of this program to a broad range of important data-driven programs in both the scientific and government communities. Specifically, the ability to not only manage equipment and data within an individual laboratory but also to securely access broad ranges of associated data, manage tasks, and coordinate projects across laboratories within a network would have a significant impact on other initiatives of interest for both basic researchers and the DOD, including projects supported by the Transformational Medical Technologies Initiative (TMTI) program. As a result, a major outcome of the midcourse assessment was the identification of the need for additional resources to advance the work of the IT team. We are currently working to identify other possible sources of support for this part of the project.

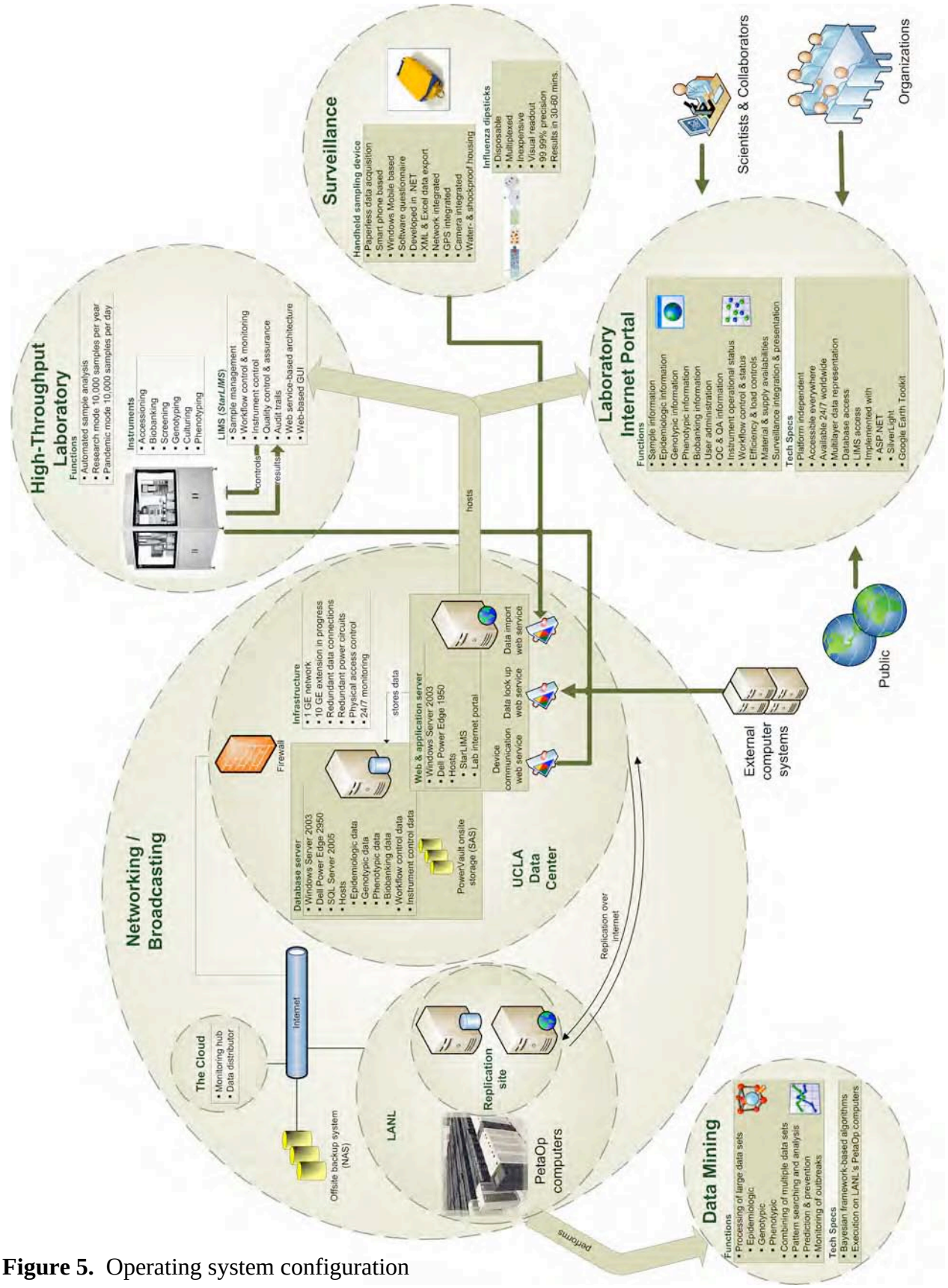

Figure 5. Operating system configuration

C. Ongoing Work in OY2 on Task 2. The IT Manager (Alexander Roth) has been closely involved in the development of the IT infrastructure for the laboratory inclusive of specifications for the various automated systems, overall laboratory workflow, implementation of StarLIMS, and establishment of network security architecture. He will continue to serve as the laboratory IT and security manager at UCLA. We are currently working to implement the data mirroring and server failover functionality, which will improve overall data and operation safety. Development of the laboratory network security concept needs to be finalized. At LANL, the continued IT work includes: (1) work on IT specifications for the *Automated Culturing System* (now completed), *Automated Archiving System* and *Automated Screening System*, (2) work on webservices for the *Automated Archiving, Accessioning, and Genotyping Systems*, (3) work on server high availability and fault-tolerance, definition of the architecture for the web-OS and (4) work on IT security issues for the lab and handling of bio-threat pathogen data. Team members at UCLA are working together with UCLA network engineers on the development of laboratory network security concept. In addition, specifications of the laboratory specific workflows need to be finalized. Currently, a draft workflow document is being reviewed interactively by members across various branches of the team. The resulting modified workflows will be used to optimize both individual automated systems and integration of these systems within the overall laboratory. During OY2, we will work to customize StarLIMS by implementing these workflows and other laboratory-specific requirements. We will continue to develop software components to integrate the *Automated Genotyping System* with StarLIMS. We must also add functions for error handling and overall audit trail and test these components. We will also continue to work on the core functionality of the web portal for data integration, analysis and presentation. We are finalizing software components for data integration and presentation. The overall operating system architecture and function is shown in Figure 5 (previous page).

Task 3: UCLA. Upgrade ~2,000 sq. ft. of floor space to BSL3+ and/or BSL3-Ag specifications for housing high-throughput automated systems.

A. Summary of original proposed scope of work for Task 3. In the original proposal (FY06, Initial Year), we proposed to upgrade containment space for the project to BSL3+ and/or BSL3-Ag specifications. This space is situated in the California NanoSystems Institute (CNSI). The space will facilitate the flow of numerous samples, reagents, supplies, waste and information. Highly pathologic avian influenza (HPAI) is a Select Agent that must be handled in biosafety containment with chain of custody. The original proposal specified an initial focus on HPAI, which would expedite preparedness for other Select Agents. We designated UCLA to accomplish this task with a chosen architecture, engineering and planning vendor (CUH2A) that specializes in laboratory work.

B. Mid-course Assessment and Progress on Task 3. Given the needs of the project and the funds available, we determined that the space in the CNSI that was assigned to the high-throughput laboratory program would be upgraded to BSL3e (as opposed to BSL3-Ag) specifications. In particular, because the cost of upgrading the facility to BSL3-Ag specifications included the requirement for concrete enhancements which were not achievable based on the floor loading capacity of the CNSI building and because there were no plans to conduct research on large animals within the facility that would require BSL3-Ag containment. Therefore, a strategic decision was made to upgrade the space at UCLA to BSL3-enhanced (BSL3e) specifications. CUH2A designed the facility upgrade (see views and drawings of facility in **Figures 6 - 14**). CUH2A, a premier architecture engineering and planning services firm for science and technology facilities (www.hdrcuh2a.com/), was selected as the design firm for the facility upgrade pursuant to an open search in which statements of qualifications were solicited from qualified firms. Prior to this project, CUH2A had designed over 50 high containment BSL-3 or higher level facilities. Throughout the upgrade process, they have worked with Capital Programs and the Project Team at UCLA to ensure that the facility is upgraded to required specifications. The upgrade work was performed by PCL Construction Service, Inc., an award-winning general contractor, which consistently has ranked in the top 25 General

Contractor Companies in the United States since 1995 and currently is ranked #7 by Engineering News Record Magazine (http://www.pcl.com/AboutUS/Awards.aspx). PCL was selected as the lowest bidder on the project out of a group of contracting companies that had been prequalified for the project based on their availability and expertise.

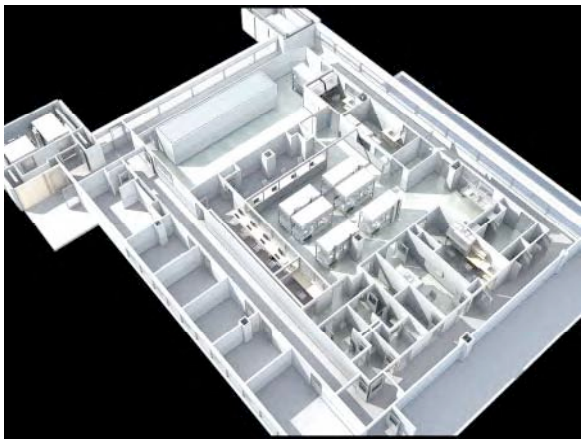

Figure 6. Three-dimensional rendering of the upgraded BSL3e facility in the CNSI at UCLA provided by the architectural/design firm, CUH2A.

Figure 7. Architect's rendition of the automation room of the upgraded BSL3e facility in the CNSI at UCLA provided by the architectural/design firm, CUH2A.

Approximately two-thirds of the cost of upgrading the ~6,200 square feet of space to BSL3e specifications was paid for using State of California Funds and one-third of the cost were paid for using FY06 and FY07 funds from this project. By leveraging the initial DOD investment, we were able to greatly expand the footprint of the facility, with a corresponding increase in potential capability of the program. The upgrade of the facility is essentially complete (see views and drawings of facility in **Figures 6 - 14**), with only final change orders and corrections being processed at present. Final commissioning is scheduled for December 2009, which will include emergency power transition testing. Initial assessments demonstrated that containment in the BSL3 spaces is intact and that redundant air-handler and exhaust fans function per specifications and perform in an integrated manner during failure testing assuring no loss of containment under emergent conditions. The security system is 90% complete and has been tested. Final programming of the autoclaves was completed September 2009, and the liquid effluent decontamination system is ready for certification at the time of commissioning. The UCLA Environmental Health and Safety Officer has reviewed the laboratory plans, inspected the facility, and signed the Certificate of Environmental Compliance.

C. Ongoing Work in OY2 on Task 3. We are currently in the process of installing door contacts and wiring to emergency exit doors for the security system and alert enunciator to indicate both a security and potential containment breach. Similarly, installation of visible strobe alarms for mechanical systems failure and integration with the Building Automation System (BAS) are in process. Purchase and installation of audible low airflow alarms for the Biosafety Cabinets (BSCs) in the two BSL2/BSL3 flex rooms have been ordered. Standard operating protocols to establish appropriate directional airflow between the main BSL3 ballroom and the flex rooms dependent on use of each room in a BSL3 or BSL2 mode are in development. Necessary signage to meet Select Agent Registration requirements has been specified. This signage needs to be ordered through UCLA facilities services. The Select Agent Registration Package needs to be completed and submitted to CDC. Documentation of final laboratory commissioning will also need to be submitted to the DOD to obtain clearance to perform testing using BSL3 agents.

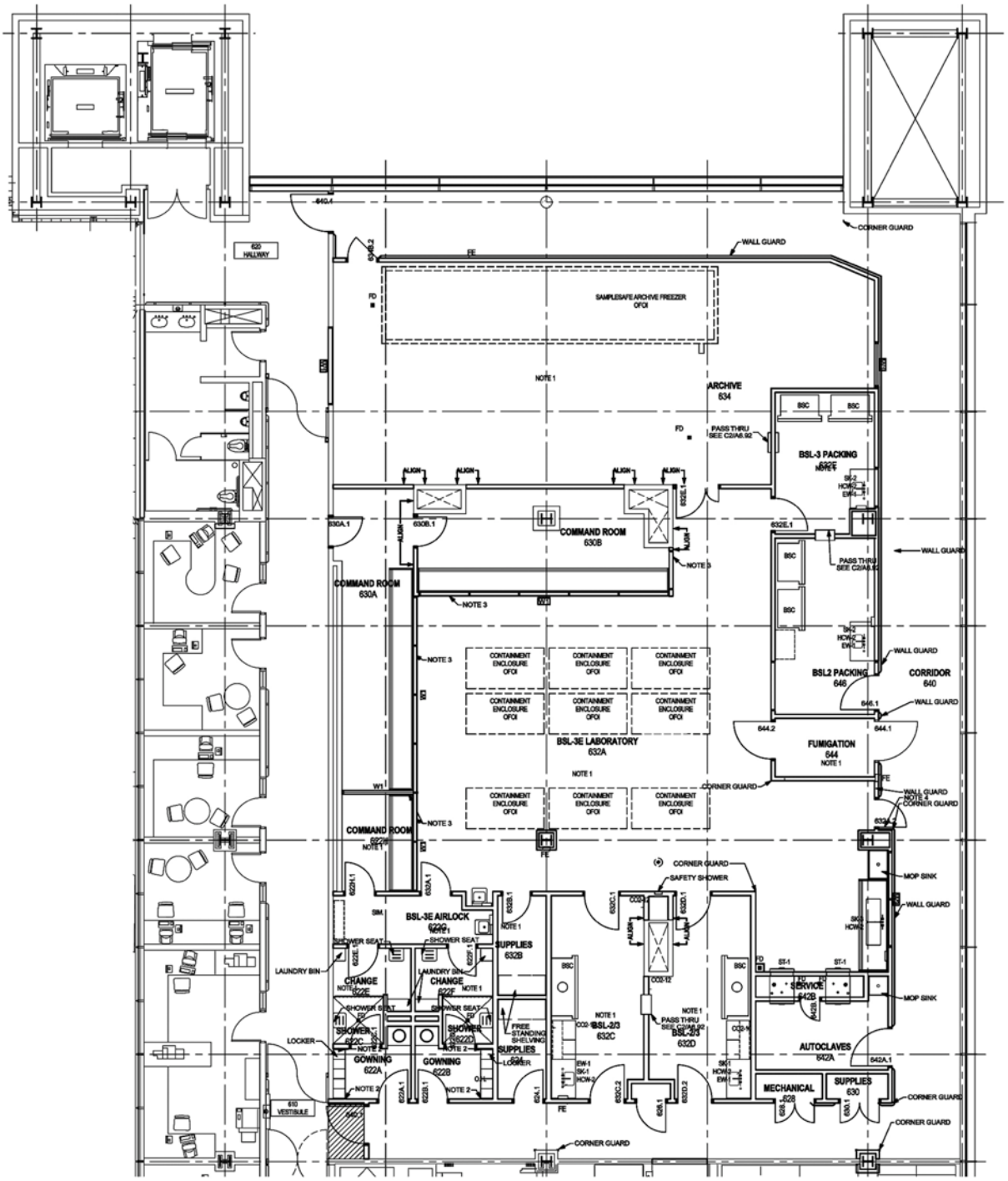

Figure 8. Blueprint for the upgraded BSL3e facility in the CNSI at UCLA designed by the firm CUH2A. The facility occupies the entire northeast quadrant of the top floor of the CNSI building. Air handling units were installed in a secure penthouse on the roof above the facility (not shown) and a decontamination tank (not shown) has been installed in a secure location in the floor beneath the facility.

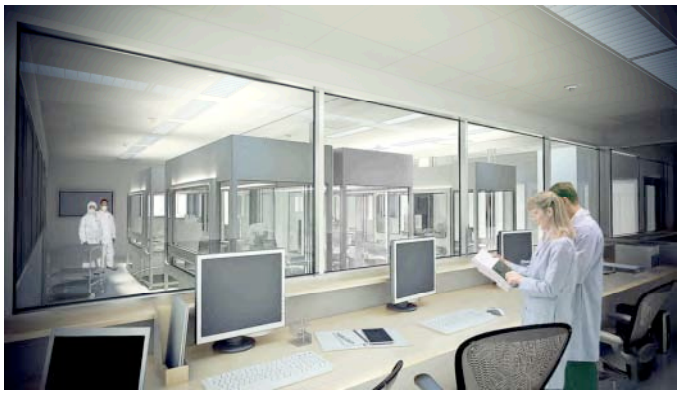

Figure 9. Architect's rendition of the view into the automation room from the command room of the upgraded BSL3e facility in the CNSI at UCLA provided by the firm CUH2A.

Figure 10. Photograph of view into the automation room from the command room of the upgraded BSL3e facility in the CNSI at UCLA, Sept. 2009.

Figure 11. Laboratory Director, Lee Borenstein, using corneal scanner to gain entrance to upgraded BSL3e facility in the CNSI at UCLA, Sept. 2009.

Figure 12. Biosafety cabinets and pass-through to Archiving room in BSL3 unpacking room in the upgraded BSL3e facility in the CNSI at UCLA, Sept. 2009.

Figure 13. Proposed site for *Automated Culturing System* in the upgraded BSL3e facility in the CNSI at UCLA, Sept. 2009.

Figure 14. Autoclaves for decontamination of materials prior to release from upgraded BSL3e facility in the CNSI at UCLA, Sept. 2009.

Task 4: LANL & UCLA. Formulate quality management program and protocols, and establish team that operates the high-throughput laboratory.

A. Summary of original proposed scope of work for Task 4. The original proposal specified that a team would be established at LANL to scale up procedures for screening and genotyping influenza viruses. We proposed that, in parallel, LANL would develop a quality control program that ensures reliability, reproducibility and error reduction. We proposed to establish a new team at UCLA to manage and operate biocontainment facilities and automated systems. To expedite this task, the LANL and UCLA teams would work together closely.

B. Mid-course Assessment and Progress on Task 4. A critical advance in the project was that Dr. Lee A. Borenstein, PhD, D(ABMM) was recruited in June 2009 to serve as the Laboratory Director for the upgraded BSL3e facility at UCLA. Dr. Borenstein has extensive experience as a Public Health Microbiologist and served previously as the Bioterrorism Response Coordinator of the Los Angeles County Public Health Laboratory, a regional National Laboratory Response Network. In addition, we designed, ordered and received the *Automated Accessioning System* (paid for with CA Office of Homeland Security funds). Operational procedures for the *Automated Accessioning System* were developed at LANL. The *Automated Accessioning System* was shipped to UCLA in November 2009 and installed in its BioCell containment module.

C. Ongoing Work in OY2 on Task 4. We have finalized system and method development for both the *Automated Culturing* and *Screening Systems*. To meet University and federal requirements to perform testing on human and avian influenza specimens or isolates, we are required to obtain approval from our Institutional Biosafety Committee and obtain Select Agent Registration with the CDC. Preparation of the UCLA Institutional Biosafety Committee Package is currently in progress. We aim to indentify an interested candidate who will be completing a National Biosafety and Biocontainment Training Program at the national Institutes of Health. For delivery and installation of both the *Automated Accessioning* and *Genotyping Systems* in November 2009 and April 2010, respectively, UCLA is also in the process of recruiting two senior level scientists, one with extensive sample processing and real-time PCR experience and one with extensive sequencing experience. Together these team members will provide technical and scientific support for both automated systems and manual processing of samples until the *Automated Screening System* is available.

Task 5: UCLA & LANL. Conduct large-scale, data-driven research on virulence, transmissibility and host range of influenza viruses.

A. Summary of original proposed scope of work for Task 5. The FY06 (Initial Year) proposal specified that UCLA and LANL would initiate a viral "pathomics" effort to understand how influenza strains are evolving and which ones pose threats. The effort will allow scientists and health officials to judge threats posed by particular subtypes and strains.

B. Mid-course Assessment and Progress on Task 5. In collaboration with CDC's Influenza Division, we are working on a set of 600 influenza B samples. We plan to generate whole genome sequences from these 600 samples and add our newly generated data to the 200 whole influenza genomes that currently exist in public access databases.

Our approach to the analysis and understanding of influenza *(i.e.,* viral pathomics) involves both hypothesis-driven and data-driven science. To begin our work, we have established an important collaboration with Dr. Nancy Cox, who is the Director of the Influenza Division at the CDC and also serves on the Scientific Advisory Board for the project. The CDC has provided 600 influenza B samples and a qualified 48 pair primer set to whole genome sequence these influenza B viruses with the *Automated Genotyping System* at LANL. The influenza B samples were chosen to represent strains that have circulated worldwide over the past 10 years. We will use the sequencing of the 600 influenza B viruses to qualify and validate the *Automated Genotyping System* prior to relocating it to the UCLA BSL3e facility.

To date, approximately 200 whole influenza B genomes have been sequenced, deposited into public databases and analyzed by previous research efforts (Chen 2008). These sequences

are mostly limited to temperate regions (primarily North America) that experience seasonal influenza epidemics. The sequencing of 600 additional whole influenza B genomes by our proposed efforts (which include samples from locations that include the tropics, where influenza circulates throughout the year) will be an important addition to information on influenza B.

Unlike influenza A viruses, which infect and circulate in many different animal species, influenza B viruses infect and circulate in humans only (Nobusawa 2006). Influenza B viruses have diverged into two lineages commonly termed "B/Victoria" and "B/Yamagata." These two lineages are immunologically distinct and vaccination with either lineage induces only weak protection against the other. It has become somewhat problematic to predict which lineage will dominate and circulate in any given year, which represents a challenge to vaccine strain selection efforts. Our hypothesis-driven and data-driven approach to influenza B analysis focuses on improving our understanding of influenza B evolution and improving vaccine strain selection efforts.

We make the following hypotheses: 1) The envelope proteins (hemagglutinin and neuraminidase) and internal proteins of influenza B are driven by different evolutionary pressures and dynamics. The envelope genes evolve (drift) according to immune pressure (selective advantage for novelty). The evolution of internal genes is driven by a need to be compatible with the envelope genes. 2) The periodic reassortment of the internal genes of influenza B enables new external genes to appear and circulate. 3) To continue to evolve and avoid evolutionary dead-ends, influenza B must periodically reassort its internal genes. 4) There is a latent pool of novel internal genes, feasibly in the tropics, that enables influenza B to periodically develop novel reassortant viruses. This implies that influenza B viruses are not well mixed on a global (geospatial and temporal) scale.

To test and refine these hypotheses, we have begun data-driven analysis of the available whole influenza B genomes. We are using this preliminary work to gain insight into existing information and better prepare for our upcoming analyses of the additional 600 whole influenza B genomes that will be generated by LANL. One principal computational algorithm that we have used for this work is based on Bayesian Markov Chain Monte Carlo (MCMC) methods and statistical models (BEAST) that yield information such as phylogeny (trees), mutation rates (for branches and gene segments) and lineage divergence (most common ancestors). These methods enable us to analyze influenza reassortment patterns (continuous, punctuated or a combination of both) and the co-evolution of segments and the internal dynamics of new reassortants, and to learn from success and failure of such reassortants (Drummond 2005). The other principal computational algorithm that we have used for this work is based on Pseudo Maximum Likelihood Methods (PMLM) that yield phylogeny (trees), genome space analyses, amino acid space analyses, and conservation/covariation information (Bruno 1996). Data-driven examinations that we will undertake pertain to: 1) segment covariation in amino acid space versus nucleotide space, 2) direct comparison of what is learned from MCMC versus PMLM methods. Depending on whether the findings from the two methods are the same or different, we will ascertain why is this the case and what is implied and/or learned from these similarities and differences.

C. Ongoing Work in OY2 on Task 5. In the next phase, our major and practical goals are to enable better predictive methods to select influenza B vaccine strains based on whole genome analyses, and to identify and/or predict potential new strains that may become dominant in coming influenza seasons. With the addition of phenotypic information on hemagglutinin and neuraminidase, we will also seek to enhance the power of the above methods. In addition, the use of other metadata (such as geospatial and temporal information and host characteristics) will enable the development of multi-scale epidemiologic models that bridge genomic, proteomic and population data in space and time. Such models will allow better prediction of influenza B evolution, spread, and effects on diverse host populations.

Task 6: UCLA & LANL. Automated Archiving System.

A. Summary of original proposed scope of work for Task 6. In the proposal submitted for FY07/FY08 (Option Year 1/Option Year 2) funding, we proposed to procure an *Automated Archiving Syst*em capable of holding 800,000 samples at –80C, that would allow for samples to be archived and specific samples to be retrieved for additional study as needed.

B. Mid-course Assessment and Progress to Date on Task 6. We have recently identified a new, commercially-available –80°C *Automated Archiving System* that is now available for sale from Hamilton Storage Technologies, Inc. (see **Figure 15**). We have placed an order for one of these systems, which is expected to be delivered in March 2010. The system is capable of automated storage and retrieval (I/O) of approximately 60,000 samples in REMP cryotubes that are generated by the *Automated Accessioning System.* We propose to use OY4 funds to purchase two additional units to allow for greater storage capacity as the facility reaches its full operating capacity.

C. Ongoing work in OY2 on Task 6. The first *Automated Archiving System* will be installed under BSL2 conditions in the new upgraded facility at UCLA during OY2.

Figure 15. *Automated Archiving System* that was purchased from Hamilton Storage Technologies, Inc using FY08 funds. Projected installation date at UCLA are March 2010.

Task 7: LANL & UCLA. Automated Culturing System.

A. Summary of original proposed scope of work for Task 7. In the proposal submitted for FY07/FY08 (Option Year 1/Option Year 2) funding, we proposed to develop an *Automated Culturing System* capable of supporting hundreds of viral isolates simultaneously, using multiple cell lines, and performing molecular (PCR) and immune (ELISA)-based assays. The *Automated Culturing System* will be capable of growing viral stocks, determining antiviral sensitivity / resistance profiles, and running viral neutralization / growth kinetics assays. The system was to be assembled to exact specifications by a chosen vendor (to be determined). An established team at LANL was designated responsibility for designing and validating the *Automated Culturing System*, which would be installed in the upgraded BSL3 facility at UCLA.

B. Mid-course Assessment and Progress to Date on Task 7. Work performed at LANL and UCLA to optimize viral extraction protocols revealed that the majority of the influenza surveillance samples which are likely to come into the facility do not have a high enough viral load to allow genotyping to be performed without prior amplification (*i.e.*, through culturing of virus extracted from the sample). As a result, a decision was made by the team that the *Automated Culturing System* should be made the next highest priority for development. Due to

the increased prioritization of the *Automated Culturing System*, significant work has been performed over the last year to develop specifications for *Automated Culturing System*, including 100% specifications for the system. In addition, as a result of the mid-course assessment, the potential need to be able to culture bacterial isolates using the *Automated Culturing System* was identified. We have made the appropriate modifications to the specifications for the *Automated Culturing System* so that vendors will include the capability to culture bacterial isolates in their proposals, if possible.

C. Ongoing work in OY2 on Task 7. At LabAutomation 2010, at the end of January 2010, members of the UCLA/LANL team met with multiple automation vendors to elicit their potential interest in submitting proposals for the *Automated Culturing System*. All the specifications for the *Automated Culturing System* have been sent to UCLA Purchasing and the RFP will be released in mid-February 2010. During OY2, we will work with the selected vendor optimize the design for the *Automated Culturing System*, which will be purchased with OY3 funds.

Task 8: LANL & UCLA. Automated Phenotyping System.

A. Summary of original proposed scope of work for Task 8. The *Automated Phenotyping System* is an integral component of the overall automation design in that it will provide phenotypic data of the type that is traditionally used for immunotyping strains of influenza, which can then be correlated with detailed genetic data (obtained from the *Automated Genotyping System*) and epidemiological data (obtained as metadata when samples are collected). The *Automated Phenotyping System* will be used to perform Hemagglutinin Inhibition (HI) and other immunotyping assays on influenza viruses. HI assays will utilize reagents and protocols developed by the CDC and the World Health Organization (WHO) for vaccine strain selection activities (Layne 2001)

B. Mid-course Assessment and Progress to Date on Task 8. Work on a detailed description of the specifications required for the *Automated Phenotyping System* has not yet been initiated.

C. Ongoing work in OY2 on Task 8. During the remainder of OY2, we will commence the design and detailed description of the specifications required for the *Automated Phenotyping System*.

***Task 9: LANL & UCLA.* Automated Accessioning System.**

A. Summary of original proposed scope of work for Task 9. The *Automated Accessioning System* is designed to handle samples as they come into the laboratory (once they have been unpacked) and prepare them for storage in the *Automated Archiving System* or for further testing or shipment to partner laboratories. The *Automated Accessioning System* divides samples into uniquely coded 0.3 ml cryotubes, hermetically seals these cryotubes, and outputs cryotubes in 96-position plates.

B. Mid-course Assessment and Progress to Date on Task 9. An *Automated Accessioning System* was purchased from Tecan Group Ltd. (http://www.tecan.com/) using Homeland Security Funds obtained from the State of California (see **Figures 16** and **17**) The *Automated Accessioning System* was delivered to and assembled at LANL in December 2007 and validated at LANL during 2008 and the first part of 2009. The *Automated Accessioning System* was shipped to UCLA in November 2009 and assembled in its HEPA-filtered enclosure purchased from BigNeat.

C. Ongoing work in OY2 on Task 9. We are currently in the process of making the *Automated Accessioning System* operational in the new upgraded facility at UCLA. The *Automated Accessioning System* will initially be validated and operated at UCLA under BSL2 conditions (BSL3 certification to be obtained by the facility in OY3). Specification of a web service-based instrument control interface for the *Automated Accessioning System* needs to be finalized.

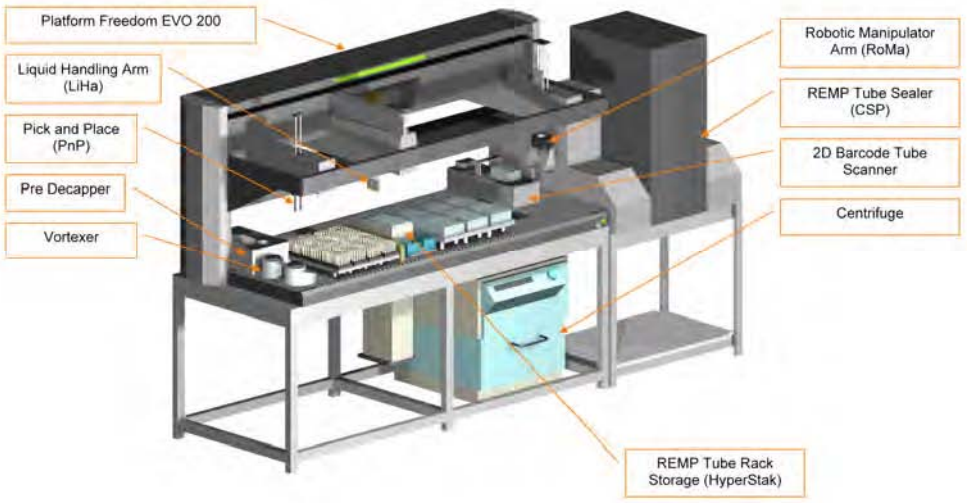

Figure 16. Schematic of *Automated Accessioning System* purchased from Tecan Group Ltd. (http://www.tecan.com/) using State of California Funds.

Figure 17. *Automated Accessioning System* installed inside its containment enclosure at UCLA.

Scientific Advisory Board (SAB). On December 11, 2008, the program's second SAB meeting was convened at UCLA. The board was composed of members from a variety of relevant disciplines. SAB members present included: Nancy J. Cox, Virginia Hinshaw, King Holmes, Colonel George W. Korch Jr., and Laurie Zoloth. SAB members absent included: Margaret Hamburg, Peter B. Jahrling, David E. Swayne, Jeffrey K. Taubenberger, and Elizabeth Wager. Those present from LANL included: Tony J. Beugelsdijk (Co-PI), Gary Resnick (Division Leader, Biosciences Division), and Mary Neu (Associate Director for Chemistry, Life and Earth Sciences). Those present from UCLA included: Dean Linda Rosenstock, Associate Dean Hilary Godwin, Associate Dean Roshan Bastani, Associate Dean Assistant Dean Kathleen Kiser, Assistant Dean Sarah Anderson, Christina Kitchen, Scott P. Layne (PI), and Thomas Smith. Overall, the SAB conveyed that significant progress had been made since the first meeting in

2007. They encouraged increased interaction with CDC, which was subsequently initiated with success as outlined above.

Mission Committee. On May 18, 2009, Tony Beugelsdijk provided a 45 minute presentation on the High-Throughput Laboratory Network to the LANS/LLNS Mission Committee in Washington, DC. The subsequent report of Mission Committee to the Board of Governors were extremely positive as follows.

> We heard a presentation on a High Throughput Laboratory Network, which is a very interesting UCLA-LANL-industry collaboration to develop new tools to rapidly characterize biological pathogens, such as the Influenza A (H1N1) virus, that could cause deadly pandemics. The system will automatically determine the genetic sequence of viruses hundreds of times faster than any method currently available. Officials could rapidly and reliably determine the strain of a virus, allowing more time for mitigation and containment in the event of a pandemic. The plan is to build into a international and interconnected capability that would provide uniformity in testing methods to enable surveillance of animal populations for the emergence of new and potentially deadly pathogens. The Committee considers this an excellent way to bring the skills of an NNSA laboratory to an important national and international need. We consider the program to be well constructed in that UCLA is providing the basic research; LANL is providing R&D, computational analysis, modeling, and validation; and industry is building the genotyping system. It is recognized that a prime system integrator will be needed for technology transfer and implementation at the national and international scale envisioned. The Committee urges that the Laboratory consider governance mechanisms for the network in the near-term and place greater emphasis on security protocols, particularly to insulate against those with malicious intent who would attempt to take advantage of the network.

KEY RESEARCH ACCOMPLISHMENTS

With FY 2006 (CLIN0001) DoD Funds

- Procured automated genotyping system. The genotyping system was delivered to LANL in May 2009 for validation and operation. System will be moved to UCLA in 2010.
- Continued development of design specifications for screening and culturing system. These efforts resulted in the advertisement of two FRPs in February 2010 and with system procurements to follow thereafter.
- Continued development of an operating system that will manage and control high-throughput laboratory systems.
- Procured Laboratory Information Management Systems (LIMS) from StarLIMS. Began integration of this software into the laboratory operating system.
- Continued and completed upgrade of new UCLA BSL3-enhanced laboratory facility. The 6,300 square foot facility is now undergoing commissioning prior to operations.
- Continued development of whole genome sequencing of influenza viruses. Established collaborations with CDC to sequence 600 influenza B samples. Continued with developing Bayesian-based methods for "pathomics" research on influenza.

REPORTABLE OUTCOMES

Manuscripts

Scott P. Layne, Arnold S. Monto, Jeffrey K. Taubenberber. Pandemic Influenza: An Inconvenient Mutation. Science 2009;323:1560-1561.

Abstracts / Presentations / Appointments

Scott P. Layne. New High-Throughput Laboratory for Molecular Surveillance of Influenza. Wadsworth Laboratory Presentation. Albany, NY. April 30 (2007).

Scott P. Layne. New High-Throughput Laboratory for Molecular Surveillance of Influenza. National Foundation for Infectious Diseases: 10th Annual Conference on Vaccine Research. Baltimore, MD. May 2 (2007).

Scott P. Layne. Melding Local, National and International Public Health Concerns. Los Angeles TEW/RAND Conference. Santa Monica, CA. May 9 (2008).

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Scott P. Layne. Near Real-Tome Surveillance of Infectious Diseases. Google/SciFoo 2008. Mountain View, CA. August 8 (2008).

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Scott P. Layne. Connecting Surveillance with the High-Throughput Laboratory Network. CNSI Membership Luncheon. Los Angeles, CA. January 29 (2009).

Scott P. Layne. How UCLA's New Global BioLab Will Dramatically Improve Response Capabilities During a Pandemic. H1N1 Virus: What UCLA Experts Have to Say. Los Angeles, CA. May 13 (2009).

Scott P. Layne , MD was appointed to serve as a member of the National Biosurveillance Advisory Subcommittee (NBAS) as authorized by Homeland Security Presidential Directive/HSPD-21. In this capacity, he serves as a special employee of United States Government, Health and Human Services, Centers for Disease Control and Prevention.

Informatics / Databases

Accession numbers for whole influenza genome sequences are deposited with BioHealthNet (http://www.biohealthbase.org) and GenBank (http://www.ncbi.nlm.nih.gov/Genbank).

Funding Applied for Based on Work Supported by this Award

See annual report for prior applications.

Principal Investigator: SCOTT P. LAYNE
Title: High-Throughput Automated –80C Biobanking Instrument for Emerging Infections
Funding Mechanism/Agency: NIH/NCRR High-End Instrument Grant Program (S10)
Cost: $1,961,908
Duration: 1 year (2009 - 2010)
Brief Synopsis: We request an automated –80C sample storage and management system configured with redundant cooling for sample protection for high-throughput infectious disease research. This biobanking instrument will serve as an indispensable resource for nine NIH-funded projects on emerging infectious threats and origins of major human infectious diseases. The award was not scored to receive funding.

CONCLUSION

Our program has made substantial progress in accomplishing all nines tasks in the Statement of Work for FY06 (first year) and for FY07 (OY1) and FY08 (OY2) as follows. 1) The *Automated Genotyping System* has been delivered to LANL; the *Automated Screening System* has been 100% specified and the RFP was advertised in February 2010 by UCLA purchasing. 2) The operating system and Information Technology (IT) infrastructure are being developed in parallel with the automated systems. 3) The upgrade of floor space to BSL3-enhanced specifications is completed; facility commissioning is nearing completion and operating under BLS2 conditions will follow next; Select Agent registration and operating under BSL3 conditions will follow later in 2010. 4) A quality control management program and protocols are being developed as we define, develop and/or refine assay methods. The LANL team that validates the high-throughput laboratory systems is established; the UCLA team that operates the high-throughput laboratory is being established. 5) The analysis of influenza B samples from human surveillance was begun in collaboration with CDC. This work begins large-scale, data-driven research on virulence, transmissibility and host range of influenza viruses. 6) Procurement of the first –80C *Automated Archiving System* from Hamilton Storage Technologies has been completed and the unit will be delivered to UCLA in April 2010; development of IT/communication specifications for interfacing with the StarLIMS system is underway. 7) The development of 100% design specifications for the *Automated Culturing System* is completed and the RFP was advertised in February 2010 by UCLA purchasing. 8) Design specifications and purchasing of the *Automated Phenotyping System* will take place in OY3 and OY4. 9) The *Automated Accessioning System* was validated at LANL and delivered to UCLA in 2009; it was installed in it HEPA-filtered BigNeat enclosure; it will be tested and placed into operation in 2010.

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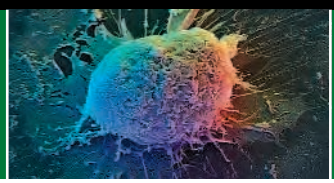
SUPPORTING / ADDITIONAL DATA

See annual report.

APPENDICES

Scott P. Layne, Arnold S. Monto, Jeffrey K. Taubenberber. Pandemic Influenza: An Inconvenient Mutation. Science 2009;323:1560-1561.

Tracy Erkkila. High Throughput Laboratory Network: Flu Lab "Blueprint. January 2010. Los Alamos National Laboratory.



LETTERS

edited by Jennifer Sills

Pandemic Influenza: An Inconvenient Mutation

SEASONAL INFLUENZA AFFECTS 10% OF THE POPULATION ANNUALLY, KILLING UP TO ONE million persons worldwide. Pandemic viruses have even greater potential for mortality. We have several defenses, including personal and public health protective measures, vaccines immunologically matched to circulating strains, and two classes of antiviral drugs (neuraminidase inhibitors and adamantane ion-channel blockers). Our preventive options are limited by viral genetic diversity and a rapid viral mutation rate. Currently, two human influenza A subtypes (H1N1 and H3N2) and two influenza type B lineages cocirculate. About 425 million doses of trivalent influenza vaccine are produced annually, enough to protect less than 7% of the world's population. In the event of a pandemic, well-matched protective vaccines against a novel agent would not be available for at least several months, highlighting the importance of therapeutic options.

By 2009, however, 98% of circulating influenza A/H1N1 strains in North America have become resistant to the frequently prescribed and widely stockpiled neuraminidase inhibitor oseltamivir (Tamiflu), and 98% of A/H3N2 strains are resistant to the adamantanes. The alternative neuraminidase inhibitor zanamivir and the two approved adamantanes amantadine and rimantadine are all in short supply, and the adamantanes have substantial side effects. Influenza therapeutic options are clearly unraveling at a time when public health officials are appropriately concerned about pandemic emergence.

Preparing for a virus storm.

The spread of high-level oseltamivir resistance in A/H1N1 strains is puzzling, as it appears to have occurred without antiviral selective pressure (*1*). Whether such levels of resistance will continue or diminish is unknown. Is high-level resistance an unfortunate byproduct of (still unknown) polygenic factors that confer viral fitness, such as balancing hemagglutinin and neuraminidase activity? Does resistance in influenza A/H1N1 imply a chance that resistance will develop in highly pathogenic avian A/H5N1 viruses, which bear the same neuraminidase subtype? Two past pandemic viruses (1957 and 1968) emerged after circulating human viruses reassorted with avian influenza viruses; emergence of a future pandemic strain by the same mechanism, but incorporating either an antiviral-resistant H1N1 neuraminidase or A/H3N2 matrix gene, is a possibility that cannot be ignored.

Pandemic planning envisions that if a virus with pandemic potential emerges, initial human-to-human transmission can be spotted quickly and contained by nonpharmaceutical interventions and by rapid community administration of antiviral agents and vaccines (*2*, *3*). If this strategy fails, a conceivable consequence, however unlikely, is accidental creation of a drug-resistant pandemic strain, a manmade analog of the feared naturally arising reassortant alluded to above.

Most national stockpiles have appropriately favored neuraminidase inhibitors (mainly orally administered oseltamivir) over ion-channel blockers (oral adamantanes) for pandemic preparedness, given the well-recognized rapid emergence of resistance to the latter when used in treatment (*4*). Now, as noted, transmissible oseltamivir resistance in human A/H1N1 strains makes this strategy problematic on many levels, including concern about efficacy in a pandemic, as well as emergence of a pandemic reassortant containing resistance genes (*1*). A complicating factor is increasing appreciation that secondary bacterial pneumonias have caused most deaths in past pandemics (*5*). Circulation of clinically aggressive community-acquired methicillin-resistant *Staphylococcus aureus* is an additional factor to be considered in planning for pandemic response. Taken together, these several developments suggest a need to continually examine and periodically reconfirm or update pandemic response strategies.

Whatever strategies are adopted, it is clear that additional anti-influenza therapeutics are urgently needed. So far, vaccines and antivirals have targeted three influenza envelope proteins: hemagglutinin, neuraminidase, and the matrix 2 ion channel protein. We need new classes of antivirals that interfere with other necessary viral processes (e.g., polymerase complex activity, interferon antagonist activity, and viral assembly). The desired outcomes of existing and future therapies (reduced severity, mortality,

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

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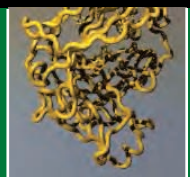
Building in flexibility

1566



Closer to combination therapies

1567

viral shedding, and transmission) should be considered with respect to both seasonal and pandemic influenza.

The unpredictable nature of influenza presents a challenge for both research and pandemic preparedness planning. Our ability to anticipate pandemic events is poor, and our anti-pandemic armamentarium is weak. In an ever-shifting landscape of influenza evolution, we need to be farsighted and forceful in optimizing pandemic response capacity.

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Romanian Expatriates Face Career Obstacles

IN HIS NEWS FOCUS STORY "REACHING FOR the stars in Romania" (21 November 2008, p. 1183), M. Enserink gives a realistic description of some important problems of Romanian science. I would like to add another important issue: Successful expatriated Romanian scientists should be encouraged to return to Romania to hold important positions, and they should be appropriately compensated for doing so. In theory, expatriated scientists are encouraged to return and take leadership roles. In practice, these scientists have trouble securing their place in the applicant pool. To qualify for consideration, the expatriated scientists must demonstrate that the position they hold abroad is equivalent to the Romanian position immediately subordinate to the open position. The legal process to determine equivalency is cumbersome, and there is no definite authority who can certify equivalence. These ambiguous requirements often serve as an obstruction to expatriate scientists.

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Reversible Exploration Not Worth the Cost

C. P. MCKAY ("BIOLOGICALLY REVERSIBLE exploration," Policy Forum, 6 February, p. 718) makes an impassioned case for so-called biologically reversible exploration of Mars. However, such a strategy will impose additional costs on an already strained program (*1*), and it is neither feasible in the context of a robust Mars exploration program nor necessary to ensure the fidelity of future in situ scientific endeavors. The concept of biologically reversible exploration is focused on potential effects of forward contamination the transport of terrestrial microorganisms to other planetary bodies. Using real options theory (*2*), we can evaluate the ability to preserve future decision paths (such as the ability to "reverse" biological incursions) with present investments [such as spacecraft sterilization and constraints put in place on "special regions" (*3*)]. An accounting of present and future scientific costs and benefits must be made to critically assess this idea. In the near term, additional costs will result from spacecraft preparation regimes, compliance, and possibly reduced mission capability due to constraints on instrumentation and landing site restrictions. The suggestion that even human exploration should achieve "biological reversibility" will impose an enormous burden on such missions in terms of both direct costs and curtailed science from restrictions on access to the subsurface. In contrast, the supposed benefits are only potential benefits, mostly in the event of terraforming, and extremely long-term in nature. The exchange of meteorite material between Earth and Mars (*4*), the flotilla of existing landed missions, and the fleet of orbiters that will eventually crash into the surface already determine both the past and near-future two-way exchange of biological material between Earth and Mars. Special regions of scientific interest on Mars do call for prudent measures to reduce contamination, but the extreme measures advocated by McKay will not yield sufficient benefits to justify their high costs.

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CORRECTIONS AND CLARIFICATIONS

News of the Week: "NSF restores data on minority Ph.D.s" by J. Mervis (27 February, p. 1161). The National Science Foundation estimates that its new policy on reporting small numbers of minorities will suppress data on 3.7% of the new Ph.D.s in the Survey of Earned Doctorates. The original story incorrectly reported that 4% of the 280 subfields would be affected.

News Focus: "Tales of a prehistoric human genome" by E. Pennisi (13 February, p. 866). The story mischaracterized James P. Noonan's mouse experiment that used an enhancer showing human specific activity. In that study (published in the 5 September 2008 issue of *Science*, p. 1346), the enhancer drove the expression of a reporter gene in the mice, but the researchers did not examine its effect on thumb development.

News Focus: "On the origin of art and symbolism" by M. Balter (6 February, p. 709). Ochre expert Ian Watts was cited as saying that there was little sign that ochre found at Twin Rivers, Zambia, was ground into powder, as needed for decoration. This incorrectly states Watts's view. Although only a small percentage of the approximately 300 pieces of ochre found at Twin Rivers show signs of grinding or other use, nearly all those that do are a dark, sparkly red. This leads Watts to conclude that they might have been preferentially chosen for symbolic purposes, although that is not certain.

Reviews: "Darwin's originality" by P. J. Bowler (9 January, p. 223). On page 226, reference 8 should read as follows: J. Browne, *Charles Darwin: The Power of Place* (Jonathan Cape, London, 2002). In reference 22, *Transmutation Notebook D* should have been *Notebook B*. Also in reference 22, two page numbers were missing: *Natural Selection*, p. 36, and *Charles Darwin's Notebooks*, p. 180.

Reports: "Observation of pulsed γ rays above 25 GeV from the Crab pulsar with MAGIC" by The MAGIC Collaboration (21 November 2008, p. 1221). The e mail address for N. Otte was incorrect. The correct address is nepomuk@scipp.ucsc.edu.

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High Throughput Laboratory Network

Flu Lab “Blueprint”

Tracy Erkkila

January 2010

High Throughput Laboratory Network

Contents

Overview

Abstract

Global infectious disease surveillance has been recognized as one of the most important approaches to combat infectious diseases, to effectively protect public health and global security. The Los Alamos National Laboratory and the University of California at Los Angeles are collaborating to develop a High Throughput Laboratory Network (HTLN) aiming to establish a high speed and high volume laboratory capability for extensive surveillance, rapid and accurate detection, and analysis of biothreat agents.

A hierarchical and modular system has been designed. The workflow consists of surveillance, sample transportation, laboratory testing, and data management and analysis. The sampling, accessioning and genotyping modules have been built, and a pilot project for the system run has been initiated. The genotyping system is capable of sequencing 10,000 full virus genomes/year, more than the total of all influenza genomes sequenced to date. UCLA will operate the first HTLN lab and serve as a model. Additional laboratories in the HTLN will be located throughout the world. The network will function much like a parallel high performance distributed computer in quickly solving a problem. The system will be housed in Biosafety Level 3-enhanced containment to enable the flow of numerous samples containing highly pathogenic avian influenza viruses and other potential select agents.

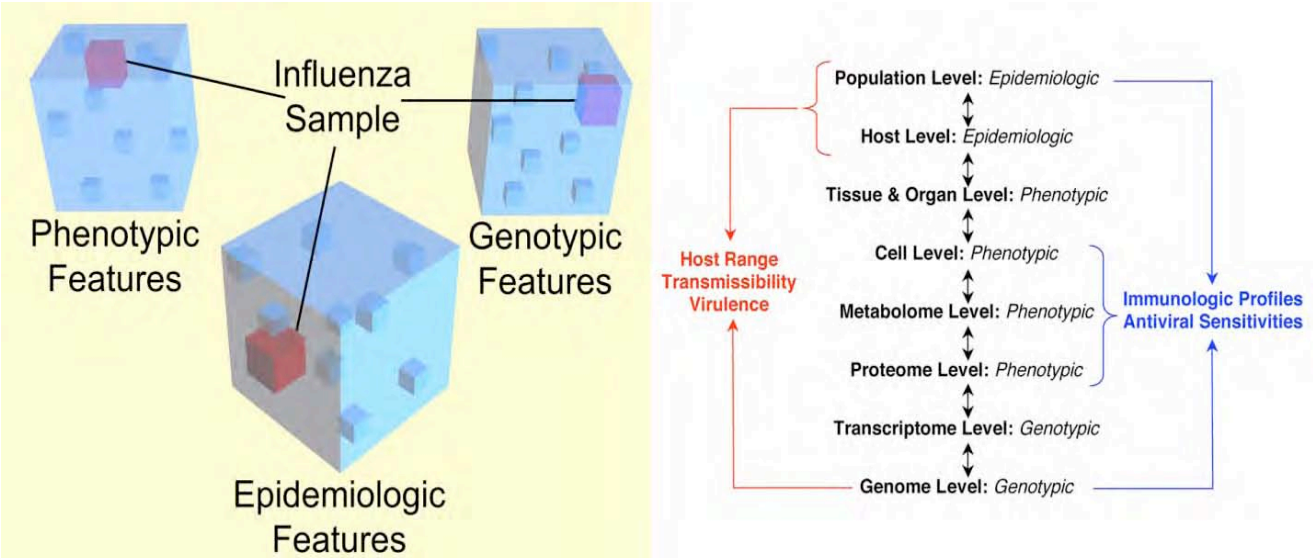


Figure 1. When fully operational, the high-throughput laboratory network would give rise to these three domains of associated data (left). These data will enable much needed epidemiologic, genotypic and phenotypic associations (right).

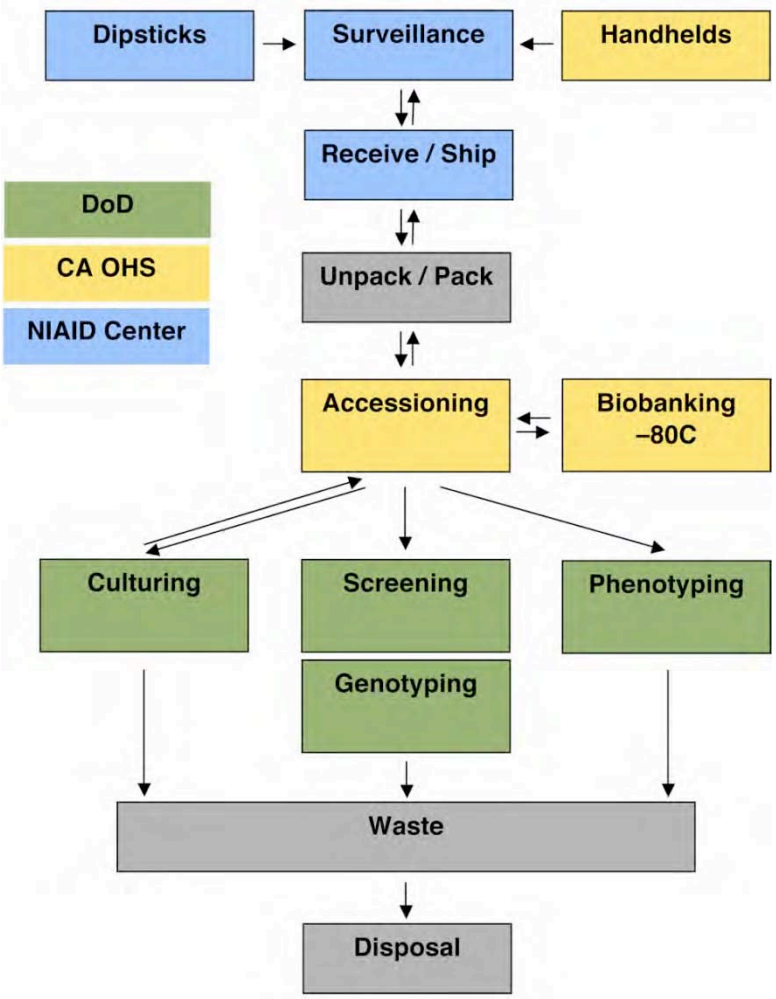


Figure 2. Automated laboratory systems shown in terms of functional relationships, funding sources and workflow. DoD funding of our program supports core automated systems, methods, and containment space upgrades. NIAID funding of the UCLA-based Center for Rapid Influenza Surveillance and Research (CRISAR) supports national and international influenza surveillance (sample streams) and dipstick development. CA OHS funding of laboratory capacity supports additional automated systems, field use handhelds and containment space upgrades that enhance high-throughput capacity.

The HTLN will provide the laboratory firepower for global infectious disease surveillance and management, becoming an international infrastructure for disease surveillance and pandemic response management. It will provide an international research and response capacity that far exceeds what is available today. It can provide information including pathogen host range and mutation rate, markers for virulence and contagion, and the mechanisms and markers of drug resistance. It also provides accurate and real time information to guide emergency outbreak control with the most effective drug or vaccine intervention strategy, and faster information for drug development or for vaccine strain selection. The rapid, large data output also provides foundational information for biothreat forensics and attribution analyses. Such a capability is critical to protect public health and societal infrastructure, and our warfighters around the world.

Figure 3. The HTLN will be a global network of High throughput laboratories, cooperatively sharing data and technology.

HTLN Goals

The near term goal for this project is to develop and pilot one or more systems to provide research capabilities (full genomic analysis) on the order of 10,000 samples per year. In the event of a pandemic, it is the intent of this project to rapidly configure the system to process 3,000 samples per day in fast screening mode.

To accomplish this, a modular architecture of hardware and software systems was designed. This architecture is scalable for increased throughput, for diversified sample types (species), and for rapid deployment.

Pandemic influenza and bioterrorism are two of the top national security threats for the United States. Influenza, in particular, would easily overwhelm National Guard response capabilities nationwide and downgrade military preparedness across all commands worldwide. A major bioterrorist attack and/or repeated smaller attacks on United States soil (Danzig 2004) would also conceivably overwhelm National Guard response capabilities nationwide, with ripple effects on military deployment worldwide.

The work accomplished by this project, to date and in subsequent years, will result in a high-throughput laboratory network (HTLN) capability (with the first node at UCLA) that can be implemented and linked with

other military, government and public institutions. The need for creating such a laboratory network has been widely recognized. The report *Making the Nation Safer* (National Research Council 2002) recommended the creation of a "global network for detection and surveillance, making use of computerized methods for real-time reporting and analysis to rapidly detect new patterns of disease locally, nationally, and — ultimately — internationally." In keeping with this report, the high-throughput laboratory network capability will be a critical addition to the DoD's chemical and biological defense and infectious disease programs.

A central element of biosurveillance must be an epidemiologic surveillance system to monitor human disease activity across populations. That system must be sufficiently enabled to identify specific disease incidence and prevalence in heterogeneous populations and environments and must possess sufficient flexibility to tailor analyses to new syndromes and emerging diseases. Integration of biosurveillance elements and other data (including human health, animal health, agricultural, meteorological, environmental, intelligence, and other data) will provide a comprehensive picture of the health of communities and the associated threat environment.

The HTLN being built in partnership by UCLA and Los Alamos National Laboratory (LANL) will achieve these functional goals by providing a platform capability (high-speed high volume processing, accessioning, biobanking, screening, genotyping, culturing) that will be coupled with the network's database (bioinformatics) to associate surveillance information from the field with laboratory data to enable rapid data-driven science and discovery. Capabilities will support analysis of traditional, enhanced, emerging, and engineered threats. When fully operational, the HTLN will protect the warfighter by providing near real-time surveillance, testing and analysis. It will also enable rapid identification and assessment of pathogens. In addition, associated data (surveillance, genotypic, phenotypic) and bioinformatic capabilities will support rational drug design approaches. In keeping with the mission of DoD programs, such as TMTI, our data-driven approach will support and enhance development of many bugs, one drug approach.

HTLN System Design

The HTLN design consists of the following systems:

1. Accessioning – provide initial sample processing, registration, and distribution into storage tubes
2. Sample BioBanking – provides -80°C storage for storage tubes
3. Extraction and Screening – provides viral RNA extraction and basic sample screening
4. Culturing – explores optimal host cell type for viral culturing, followed by scale up of viral sample
5. Genotyping – performs PCR and sequencing reactions for genotyping by Sanger sequencing
6. Phenotyping – provides screening of viral samples for various serotypes and phenotypes
7. Viral Data Analysis – provides a semi-automated analysis pipeline for the data generated from phenotyping and genotyping
8. LIMS/system integration – manages the laboratory operations, inventory, and data. Ensures quality and performance.

The eight systems will provide three domains of associated data that will enable much needed epidemiologic, genotypic and phenotypic associations for infectious diseases like influenza.

The Accessioning and Genotyping systems have been specified and assembled. The Accessioning system has been validation at LANL and shipped to UCLA for final install. The Genotyping system has been assembled and is currently undergoing validation at LANL.

The first version of the Biobank is in procurement stage.

The Extraction and Screening and Culturing systems are in the specification and pre-procurement stage.

Phenotyping is still in design stage.

Viral Data Analysis is in conceptual stage, with analysis of pilot data now underway.

LIMS/system integration is in design and implementation stage.

Figure 4 shows a high level overview of the systems depicts a logical flow of samples between the systems. The numeric annotations are the intending daily sample processing capacities for each system. The extraction and screening system is designed to handle up to about 3,000 samples per day in fast screening mode. The culturing system is designed to initiate the culturing of up to 500 new viral samples per day. The genotyping system is designed to process up to 320 viral RNA samples per day.

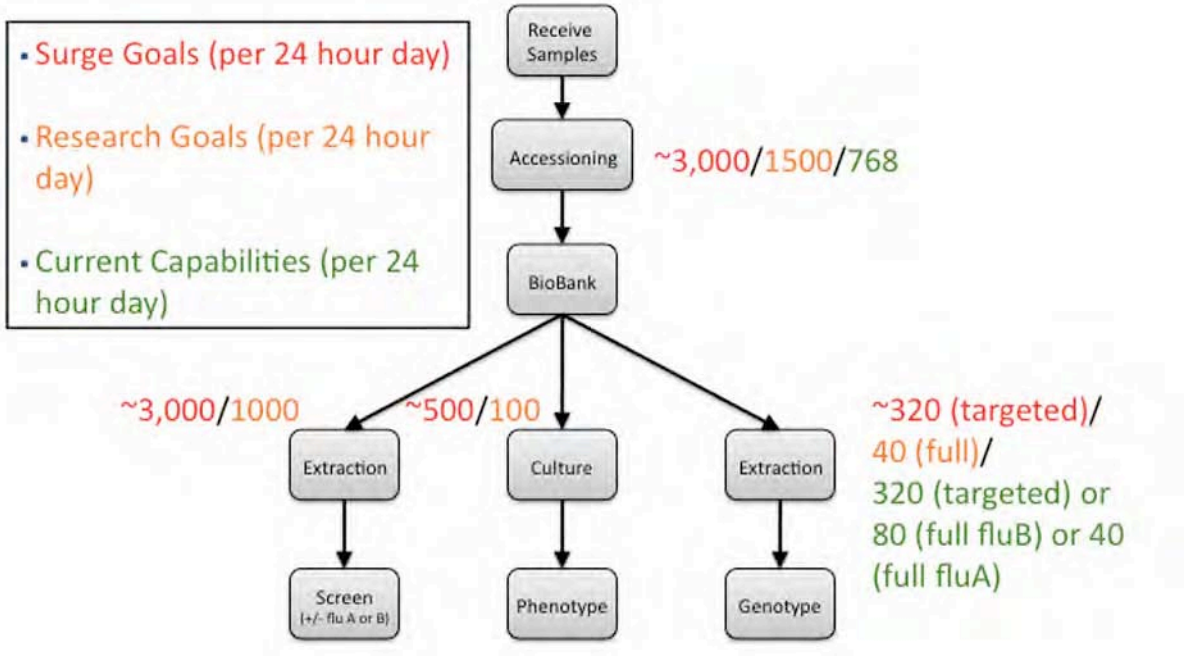


Figure 4. Overview of the HTLN system architecture, showing designed capacity in terms of numbers of samples to be processed per day in pandemic or surge mode.

The HTLN expects to receive several different types of samples that will require different types of processing and analysis. Figure 5 shows four different possible paths. Some samples may be of unknown origin and basic screening of the samples are required. Other samples may be already characterized to some degree, and only genotyping will be required. Still other samples such as avian surveillance samples may need rapid screening followed by genotyping. And finally, some samples may require a more intensive path of extraction, screening, culturing, phenotyping, and genotyping.

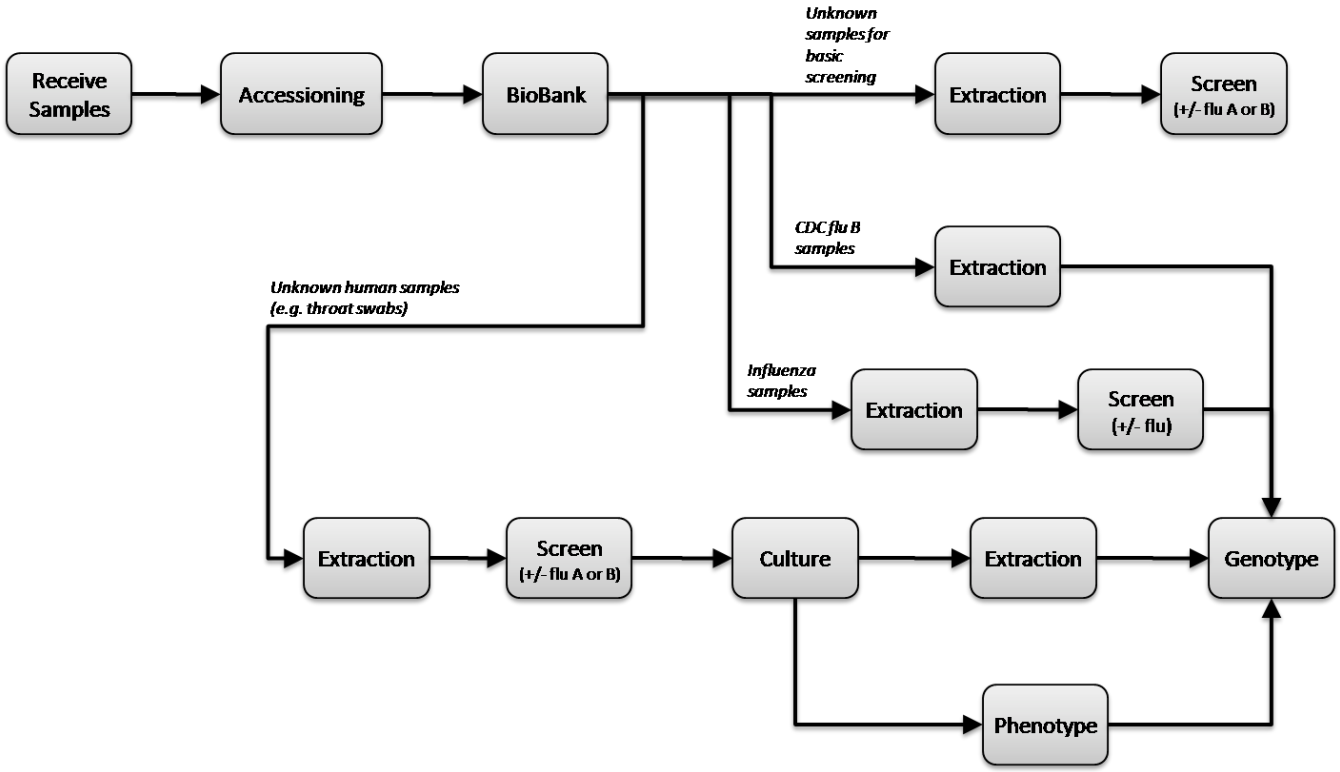


Figure 5. Overview of logical flow of samples through the HTLN. The four different paths shown here for example depict four possible sample types.

Detailed descriptions of the various systems within the HTLN follow below.

Accessioning System

The Accessioning system provides the initial registration of samples received by the laboratory into the system and the system database by transfer samples from screw-cap sample vials (2ml or 5ml) into REMP storage plates containing 96 storage tubes. Each storage tube (2ml) is individually identifiable with a 2D barcode on the bottom.

The accessioning process consists of uncapping an input vial, adding solvent to the sample, recapping vial, vortex mixing sample with solvent, centrifugation of sample, uncapping vial, aspiration of supernatant and distribution into 6 storage tubes within a REMP plate. Finally, the REMP plate is sealed with foil, foil seal cut for 96 individual tubes, and REMP plate is stacked for output to the BioBank. This sequence is shown in Figure 6.

Samples are presented to the accessioning system in racks of 32 vials. Each sample is processed and aliquoted into 6 REMP storage tubes. All transfers are tracked by the LIMS system. The mapping of vial-to-plate transfers is also shown in Figure 6.

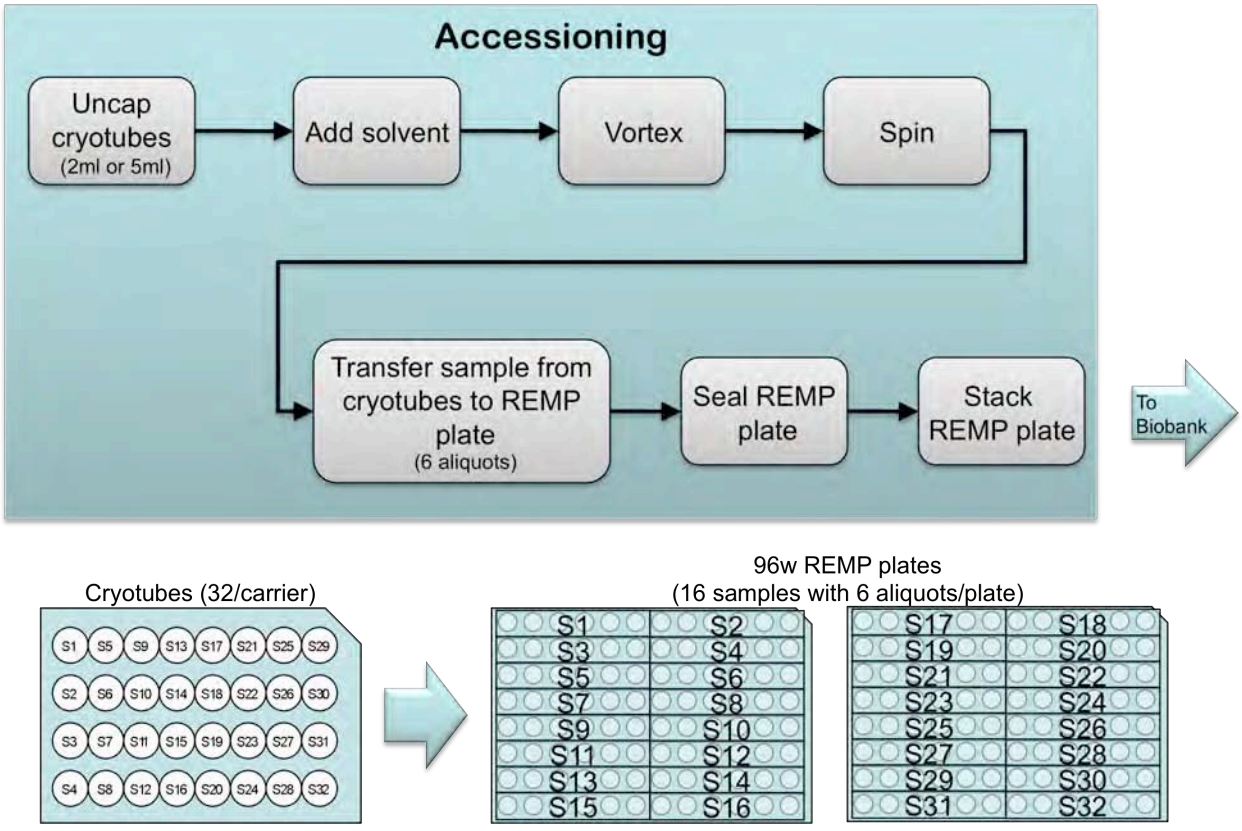


Figure 6. Overview of the Accessioning process (TOP). Container mapping from screw-top vials into 96-tube REMP plates (BOTTOM)

An overview of the system is shown in Figure 7. The system is comprised of:

1. Tecan EVO 200 8-tip Liquid Handling Arm (LiHa) with Pick and Place arm (PnP) for tube handling and Robotic Manipulator Arm (RoMa) for REMP pate handling
2. Heidolph Vortex
3. Keyence Barcode scanner
4. Vial Gripper
5. Hyperstak (Output)
6. Hyperstak (Input)
7. REMP Reatrix (2D barcode scanner)
8. Cerionix Tip Charger for liquid handling tip cleaning and sterilization
9. Hettich Centrifuge
10. Te Link plate transport conveyor
11. REMP CSP tube sealer and cutter

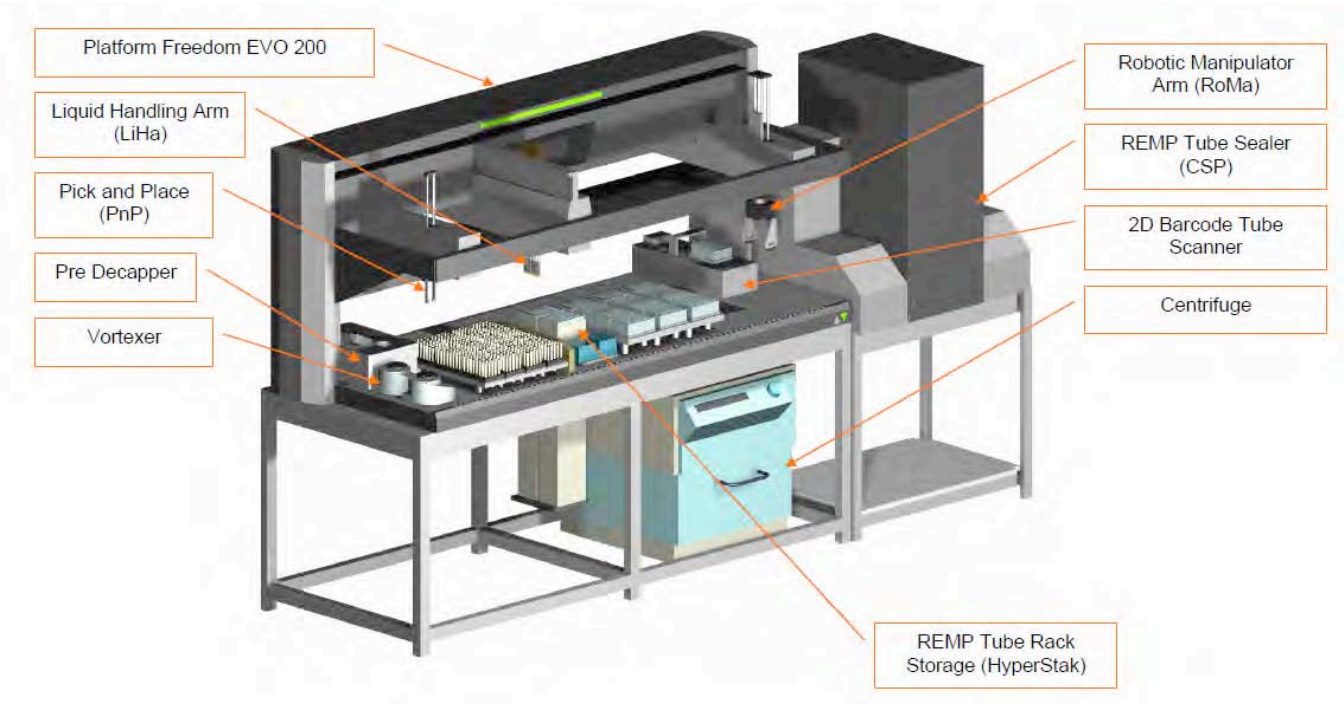


Figure 7. Layout overview of accessioning system, showing the various components.

A photo of the final system is shown in Figure 8. The system is shown in the laboratory at LANL undergoing system validation and testing. A photo of the system as installed in the lab within its safety enclosure at UCLA is shown in Figure 9.

The accessioning system is expected to provide the processing of 32 sample vials into 2 REMP plates in about 3 hours. This process is mostly time limited by the uncapping and capping function required to access the vials. With this throughput, the system can process about 96 samples per 12 hour shift.

Figure 8. Accessioning system, consisting of a Tecan EVO and a REMP plate sealer/cutter. Plate stacker and centrifuge are under the Tecan deck.

Figure 9. Accessioning system installed inside containment enclosure at UCLA

Concerns

1. The Hyperstak device that holds completed REMP plates after accessioning, is not temperature controlled. This may jeopardize the integrity of the samples if they are held at ambient conditions for prolonged periods before transfer to the BioBanking system for storage at -80°C.

2. In order to accommodate the designed demand of the HTLN pilot system, some increase in efficiency will be required. The first obvious way to increase capacity is to "clone" the accessioning system.

   Increased efficiency may also be accomplished with this system by utilizing some type of pierceable cap on the sample vials. This would minimize the time incurred by uncapping and capping the vials. This capping/uncapping time is a significant portion of the overall processing time per sample.

3. There are several additional protocols that may be necessary in order to support other type of sample accessioning. These protocols will either need to be written by Tecan or by HTLN project personnel.

BioBanking System

Samples stored in the laboratory for processing need to be stored at -80°C to maintain sample integrity. Samples will be stored in 96 tube REMP plates. As requests for samples are made, the system will reformat or cherry pick tubes and output REMP plates with desired samples and configuration of samples.

An overview of the BioBank operations are shown in Figure 10. Received samples can be consolidated from many input plates into a single storage plate, stored as-is, or reformatted into a variety of configurations as necessary. Samples requested for output can be delivered as-is, cherry picked from many source plates, or reformatted as necessary.

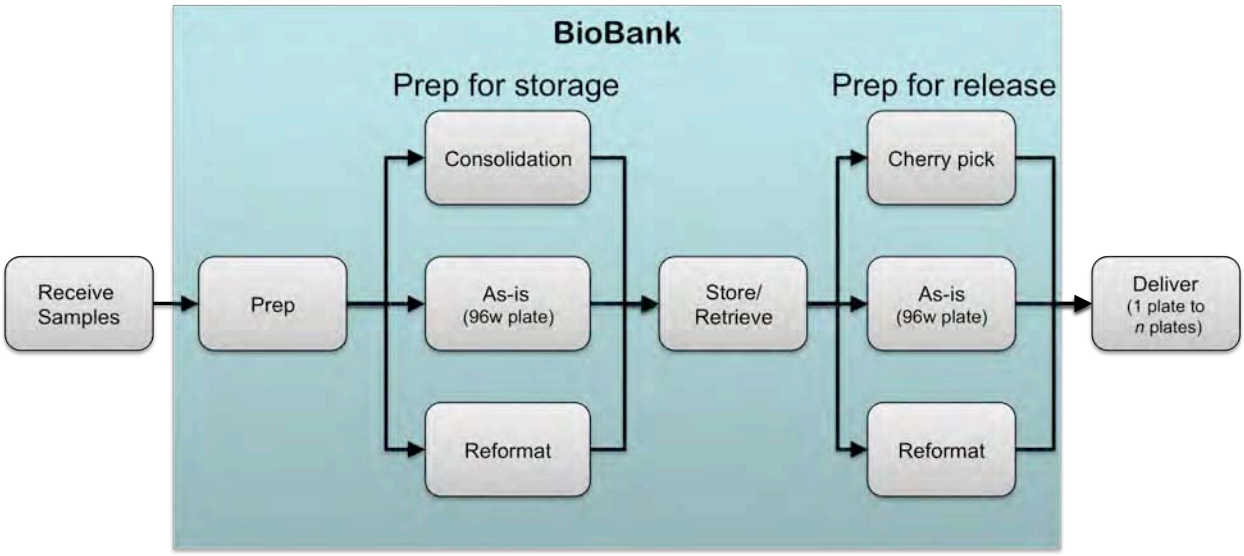


Figure 10. Overview of BioBank operations

Figure 11 and Figure 12 show example STORE and RETRIEVE scenarios.

In the example STORE scenario, the input plates are from the accessioning system and contain 6 aliquots of each viral sample. Two aliquots are transferred to a destination plate. This destination plate will contain up to 48 unique viral samples, and will be suitable for use in requesting samples for the culturing or extraction/screening systems. All plates are stored in the BioBank until needed. This type of STORE operation is intended to decrease access time in the future when samples are requested for culturing or extraction/screening.

In the example RETRIEVE scenario, the input plates are the 2-aliquot per plate containers created in the above STORE scenario. The output is a plate with 96 unique viral samples, ready for input to the culturing or extraction/screening systems.

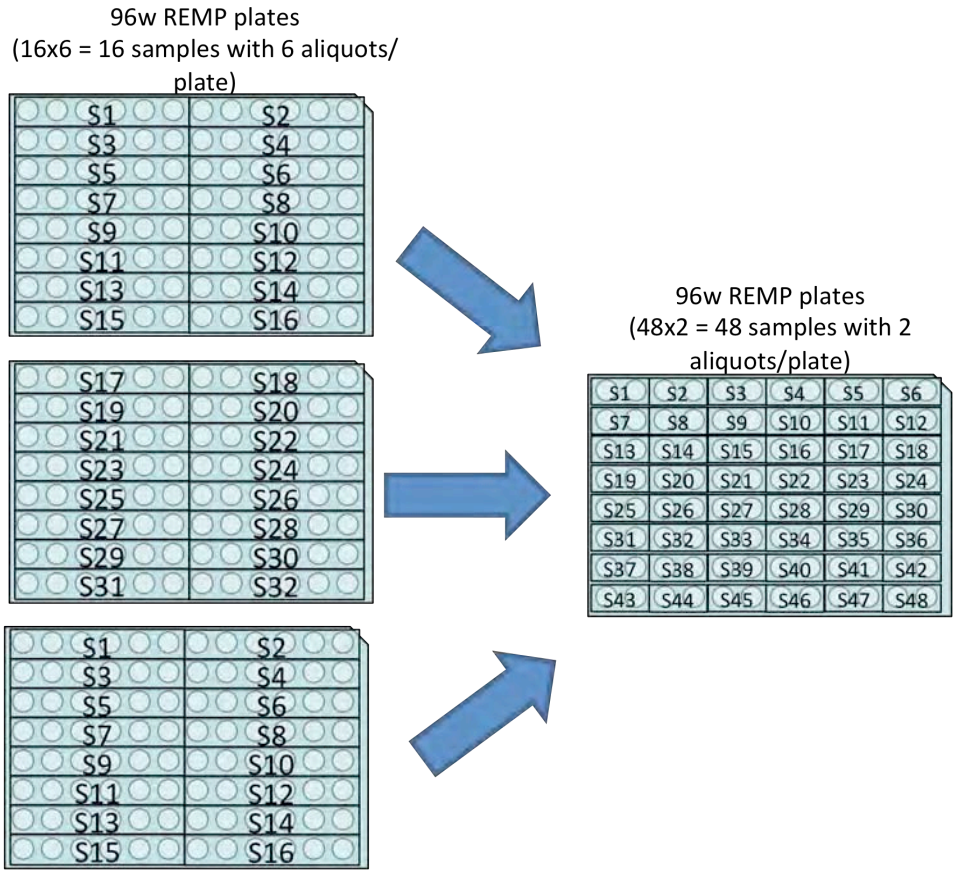


Figure 11. Example STORE scenario that transfers 2 tubes per unique sample into a 96-well REMP plate. This two-sample per virus plate can then be used as the starting plate for culturing or extraction processes.

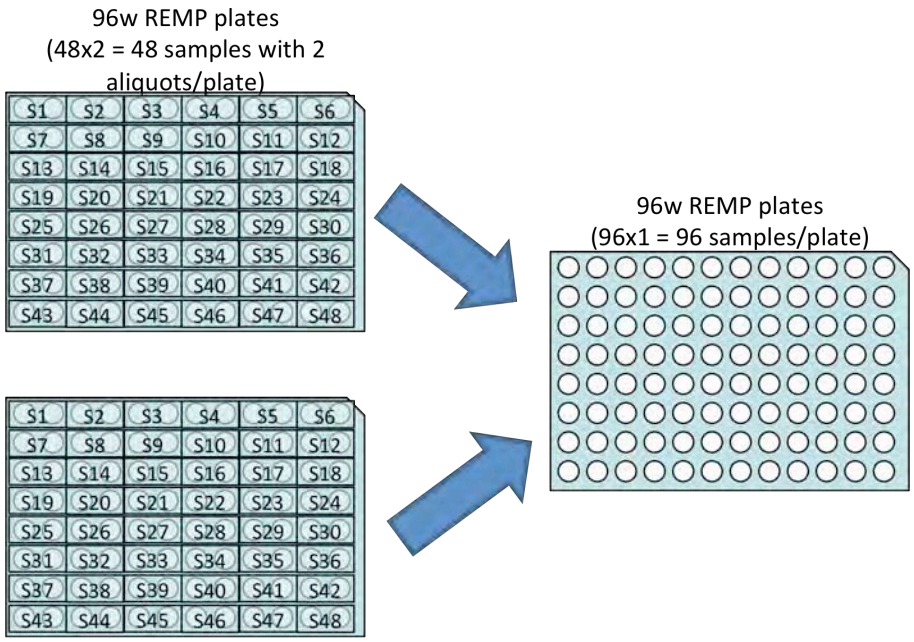


Figure 12. Example RETRIEVE scenario that involves transferring 1 aliquot from a 2-aliquot plate into a plate ready for culturing or extraction/screening.

Several commercial storage systems were considered, and the Hamilton Sample Access Manager was chosen for the pilot system. The Hamilton storage system can accept one 96-tube REMP plate as an input or output. Storage capacity is approximately 600 REMP plates. Each store/retrieve operation takes approximately 50 seconds. During any tube transfer operation (reformatting or cherry picking), one source plate and one destination plate are used at a time. An example tube picking operation of picking 16 tubes per REMP plate across 6 source plates (96 tubes in total) into a single destination plate will take approximately 1 hour. This is a typical operation of creating an input plate ready for culturing or extraction operations.

A photo of a typical Hamilton system is shown in Figure 13.

Figure 13. Hamilton -80°C Sample Access Manager (SAM) storage system.

Concerns

With a combined demand of up to 40 REMP plates per day (4,000 samples or REMP tubes per day), multiple systems will be ultimately required to keep up with throughput demands of the HTLN pilot system. In any case, with a 600 REMP plate per system storage capacity, multiple systems will be required to accommodate the storage needs of the HTLN.

Larger capacity storage systems with quicker turnaround times should be considered for the future.

Extraction and Screening System

The Extraction and Screening system is currently under design and will soon go out for bidding by vendors under and RFQ from UCLA. This system is being designed to handle approximately 10,000 extraction and screenings per day in pandemic or surge mode, and about 500 per day in research mode.

Figure 14 below shows an overview of the functional workflow of the extraction and screening system and its connection to the BioBank and Genotyping systems. The letter annotations (A thru C) indicate the protocols supported by the system.

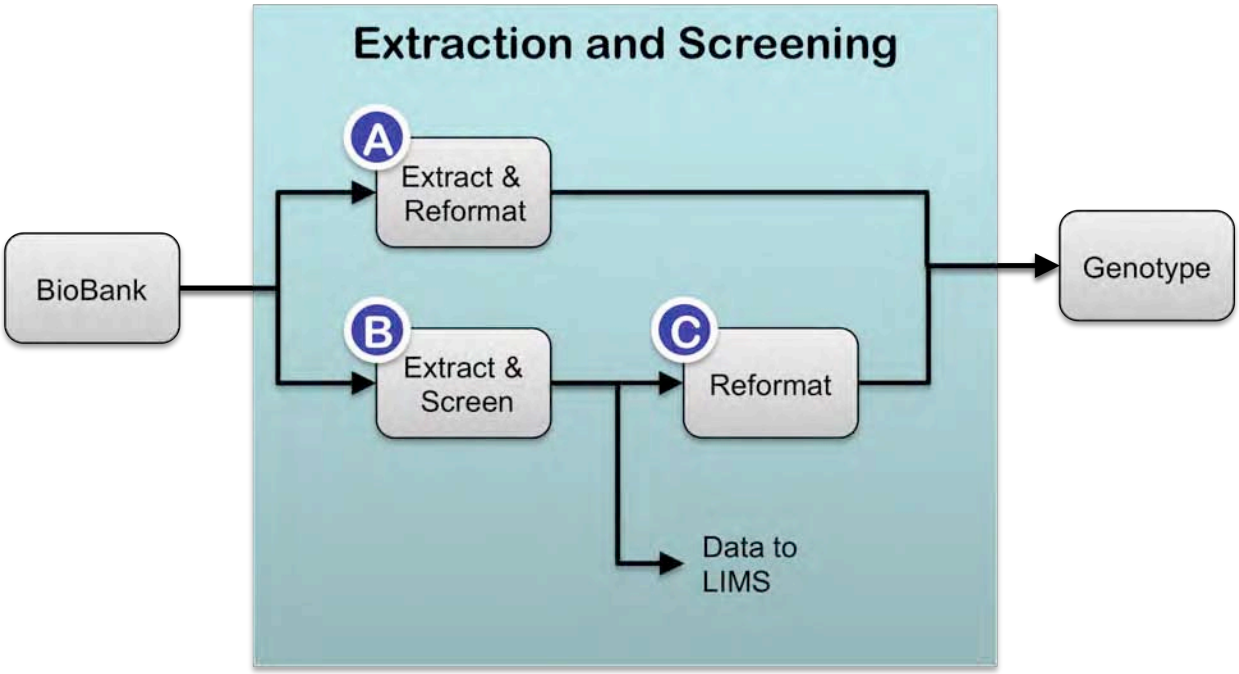


Figure 14. Overview of the Extraction and Screening System operations

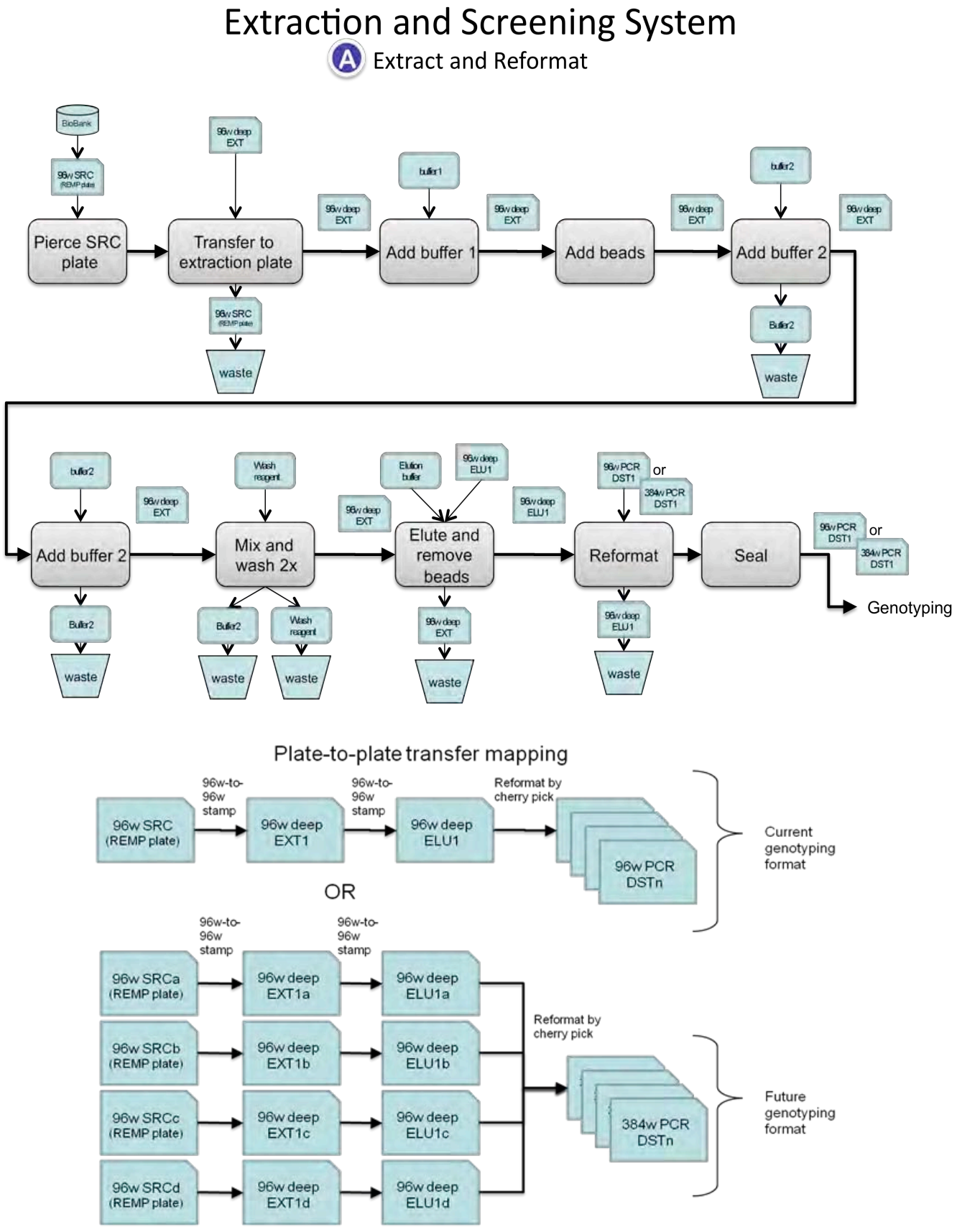
The Extraction and Screening system is logically divided into 3 protocols.

A. Extract & Reformat – this protocol accepts as input samples from the BioBank in REMP plates with up to 95 sample tubes each. RNA is extracted from the input samples using magnetic beads. The extracted RNA is reformatted into 96-well or 384-well plates for processing by the Genotyping system.

B. Extract & Screen – this protocol accepts as input samples from the BioBank in REMP plates with up to 95 sample tubes each. RNA is extracted from the input samples using magnetic beads. An aliquot of the extracted RNA is screened by RT-PCR, and the remaining RNA is temporarily stored.

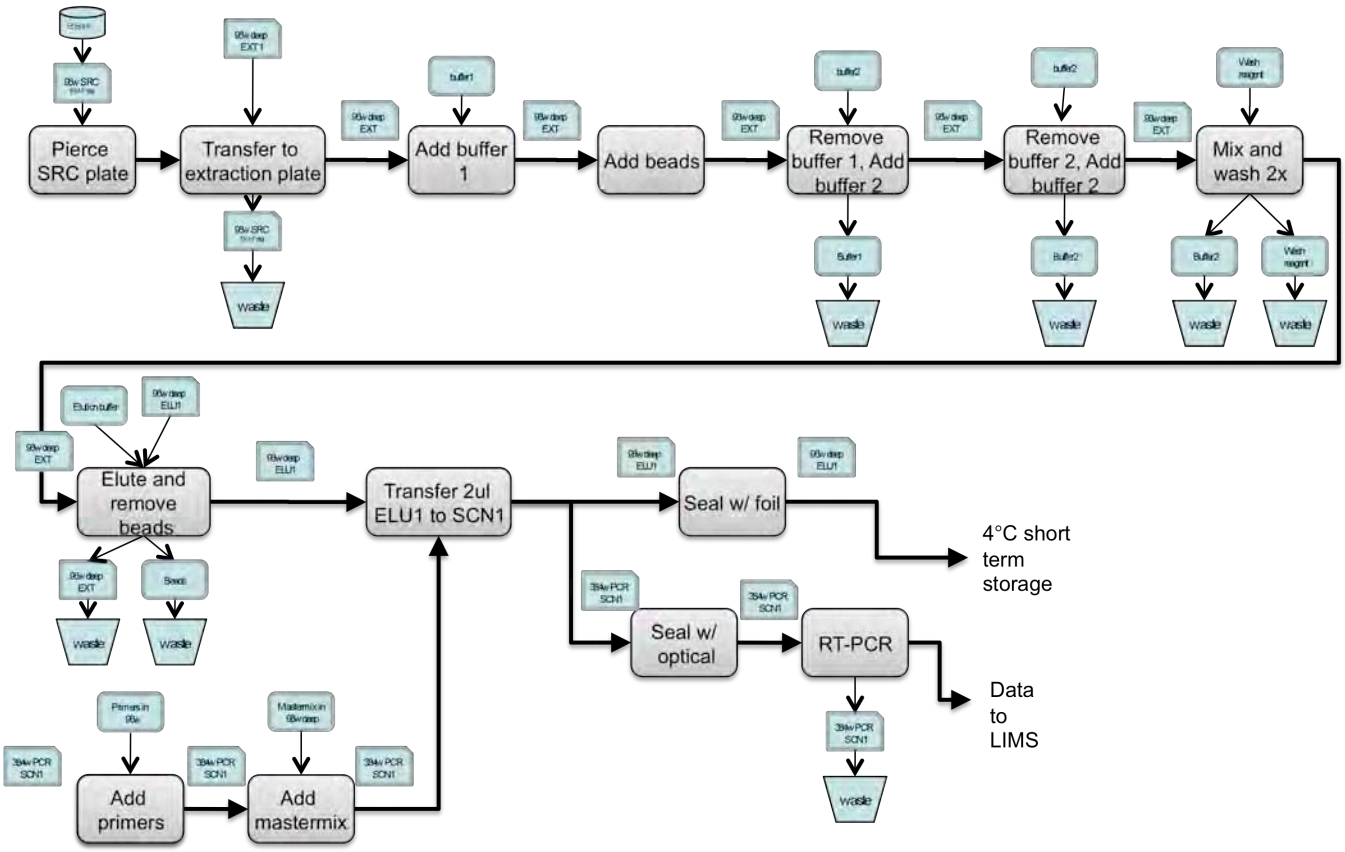
C. Reformat – the protocol starts with the temporarily stored RNA from Extract & Screen, and reformats it into 96-well or 384-well plates for processing by the Genotyping system.

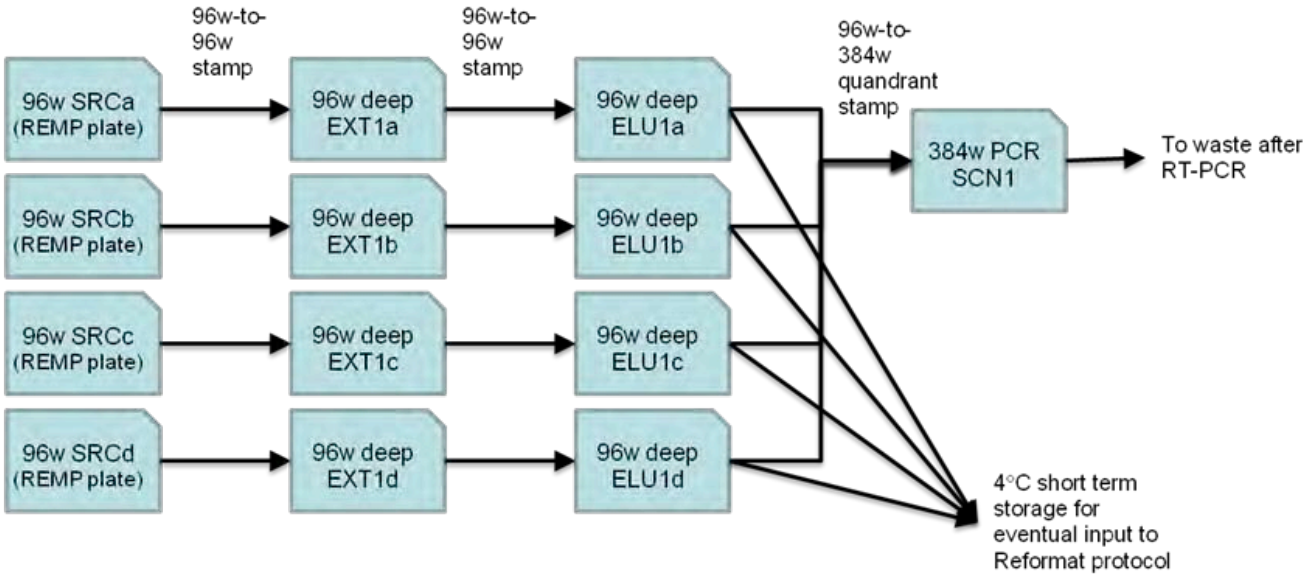
These three protocols and their associated plate-to-plate mapping are depicted in detail in the following figures.


Extraction and Screening System
Extract and Reformat
BioBank
96w SRC (REMP plate)
Pierce SRC plate
96w deep EXT
Transfer to extraction plate
waste
buffer1
Add buffer 1
Add beads
buffer2
Add buffer 2
Buffer2
Wash reagent
Mix and wash 2x
Elution buffer
96w deep ELU1
Elute and remove beads
96w PCR DST1
384w PCR DST1
or
Reformat
Seal
Genotyping
Plate-to-plate transfer mapping
96w SRC (REMP plate)
96w-to-96w stamp
96w deep EXT1
96w deep ELU1
Reformat by cherry pick
96w PCR DSTn
Current genotyping format
OR
96w SRCa (REMP plate)
96w SRCb (REMP plate)
96w SRCc (REMP plate)
96w SRCd (REMP plate)
96w deep EXT1a
96w deep EXT1b
96w deep EXT1c
96w deep EXT1d
96w deep ELU1a
96w deep ELU1b
96w deep ELU1c
96w deep ELU1d
384w PCR DSTn
Future genotyping format

Extraction and Screening System

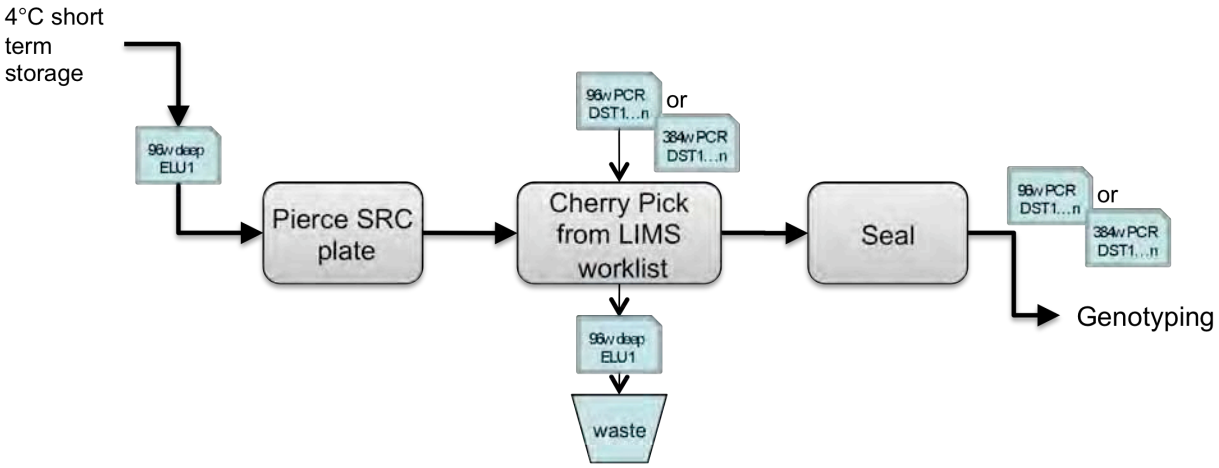
B Extract and Screen

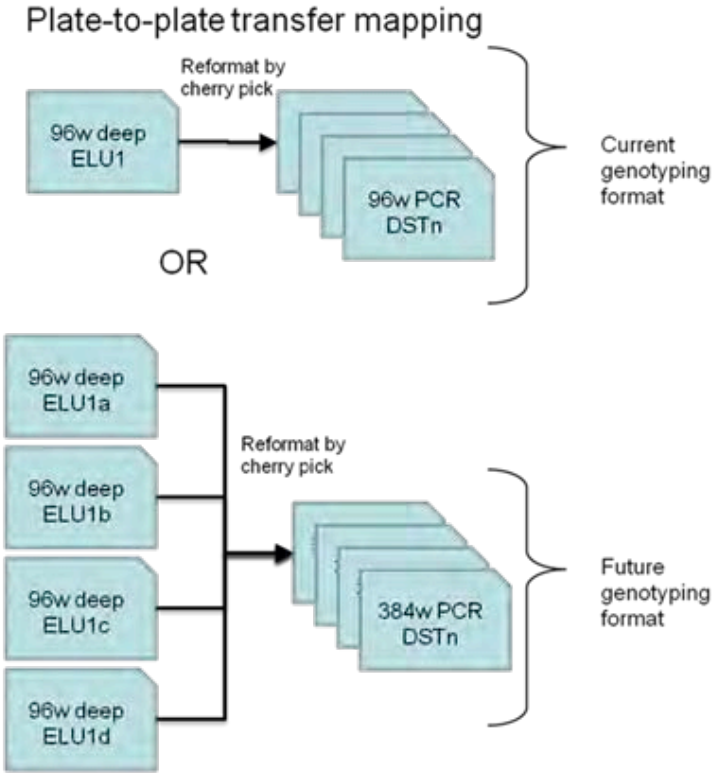




Extraction and Screening System

Reformat





System Specifications

The system specifications for the RFQ are summarized below.

Purpose and Functionality Requirements

1. Establish a nucleic acid extraction and screening system using a fully-automated system requiring minimal human input of labor or handling.
2. While influenza is our model organism for this system, it should also be flexible and compatible with BioWatch, and public health methodologies and reagents.

Operational Requirements for the System:

1. Fully automated, with minimal human intervention
    a. RNA or DNA extraction
    b. An integrated system with components to screen samples for pathogens using Real-time PCR
2. Data transfer between system and Laboratory Information Management Systems such as StarLIMS using web services (see separate web services document).
3. Flexible construction, allowing for change and exchange of pieces of equipment when required and/or as technology evolves
4. Easy access to all integrated devices for service and maintenance
5. Minimize downstream costs of consumables, including tips, reagents
6. Able to withstand cleaning with 10% household bleach and vaporized hydrogen peroxide
7. System shall provide uninterruptible power for a minimum of 5 minutes to provide for a graceful shutdown.
8. In the event of power loss, system will finish current operations and pause.

Safety Requirements

1. This system is expected to run with live pathogens on the deck. Personnel, product, and environmental protection needed. Enclosure with directional airflow and HEPA-filtered exhaust for the system will be required.
2. Emergency stops
3. Interlocked access doors to system enclosure
4. Clean to dirty workflow and airflow
5. Waste should be stored in such a manner that it does not pose a safety or aerosol hazard to personnel. Waste should also be stored so that it does not pose a contamination hazard to the product or workspace.
6. Access to refill reagents and consumables should accomplished without breaching safety containment enclosure.

Required System Components:

1. Magnetic bead extractor(s) with a maximum cycle time of approx 30 minutes per 96 well plate.
2. Refrigerated storage/retrieval carousel

a. 4°C storage for labile reagents
b. 4°C storage for elution plates before screening
c. Technician must be able to access/refill reagents without having to "enter" the HEPA-filtered enclosure

3. Plate storage, transfer
    a. Plate Hotel capable of presenting a minimum of five different types of microwell plates (and additional slots for tips if needed)
    b. Plate transfer to/from components
    c. Technician must be able to access/refill plates or plastic consumables without having to "enter" the HEPA-filtered enclosure
4. Liquid handling
    a. To transfer samples to extraction plates (foil-piercing ability required)
    b. To add or remove reagents for magnetic bead extractors
        - Fill plates with bead suspension
        - Fill plates with lysis buffer
        - Fill plates with wash solution
    c. To export extracted nucleic acids in the format specified by the genotyping system
        - 6 formats based on primer design
    d. To maintain magnetic beads in suspension
9. Plate barcode reader for microplates
5. Plate sealers
    a. sealing for Real-time PCR plates (optical film)
    b. sealing for extraction plates (piercable foil)
6. Real time PCR
    a. Five color Real time RT-PCR, capable of handling 384-well plates
    b. Liquid handling needs:
        - Positions for 96 deepwell mastermix plates defined by barcode (4°C)
        - Position for 96 well PCR type primer+probe plates defined by barcode (dark, 4°C)
    c. PCR instrument will need to be swappable with a BioWatch compatible instrument when needed (currently 96 well ABI 7500)

Reagent and Disposable Material Requirements:

1. Buffer 1 (Guanidium-based)
2. Buffer 2 (Alcohol Wash &/or guanidium-based)
3. Bead slurry
4. Wash solution
5. Elution buffer (For RNA organisms, may be water, for DNA organisms, must be pH neutral)
6. Extraction plates/tubes as required by the system
7. Tips if needed
8. Real-time PCR Plates
9. Eppendorf PCR plates for export of NA - Eppendorf cat # 951020624 twin.tec 96-well skirted and 951020702 384-well (or similar weight plates)

10. Agilent Pierceable Aluminum Seal cat # 06644-001 (or similar gauge seal)

Other Details in Technical Requirements:

1. Volume: 1 µl – 1 ml handling
2. Input of 0.30 ml REMP tubes from accessioning system or archive
3. Output of foil-sealed 96 or 384 well plates from nucleic acid extraction to genotyping system

Throughput Estimate:

~10^4 Real-time-PCR extraction and screening reactions per 24 hour day (in full surge capacity) 9216 sample-throughput acceptable (96 x 96 well plates).

1. For magnetic bead extraction: 30 min per 96 well plate per 24 hour day = 4608 well/day/extractor – two extractors may be necessary
2. For screening: 1.5 hours per 384-well Real-time-PCR cycle in 384 well plates = 6144 wells/day/thermocycler – this may be sufficient if not all samples are screened (for example: samples coming from culture system will have already been screened for the presence and quantity of virus)

Dimension Requirements:

1. Footprint of assembled system should be no larger than 12’ x 7’ with overall height less than 7 feet
2. 4 foot access buffer around system
3. Disassembled components should be no bigger than 44” x 84” (to fit through a standard laboratory door)

Waste:

Liquid waste will be at BSL1 level, but may contain RCRA hazardous wastes such as alcohols or phenols. Segregation of lysis buffer and wash buffer waste will be necessary to reduce waste disposal costs. Solid waste such as REMP tubes may have residual Risk Group 2 (RG2) organisms in the bottom of the wells and will need to be automatically bagged for transport to autoclave. Waste should be stored so that it does not pose a safety or aerosol hazard to personnel. Waste should also be stored so that it does not pose a contamination hazard to the product.

Culturing System

The Culturing system is currently under design and will soon go out for bidding by vendors under and RFQ from UCLA. This system is being designed to handle approximately 500 sample culturings per day in pandemic or surge mode.

Figure 15 below shows an overview of the functional workflow of the culturing system and its connection to the BioBank and Accessioning systems. The letter annotations (A thru I) indicate the protocols supported by the system.

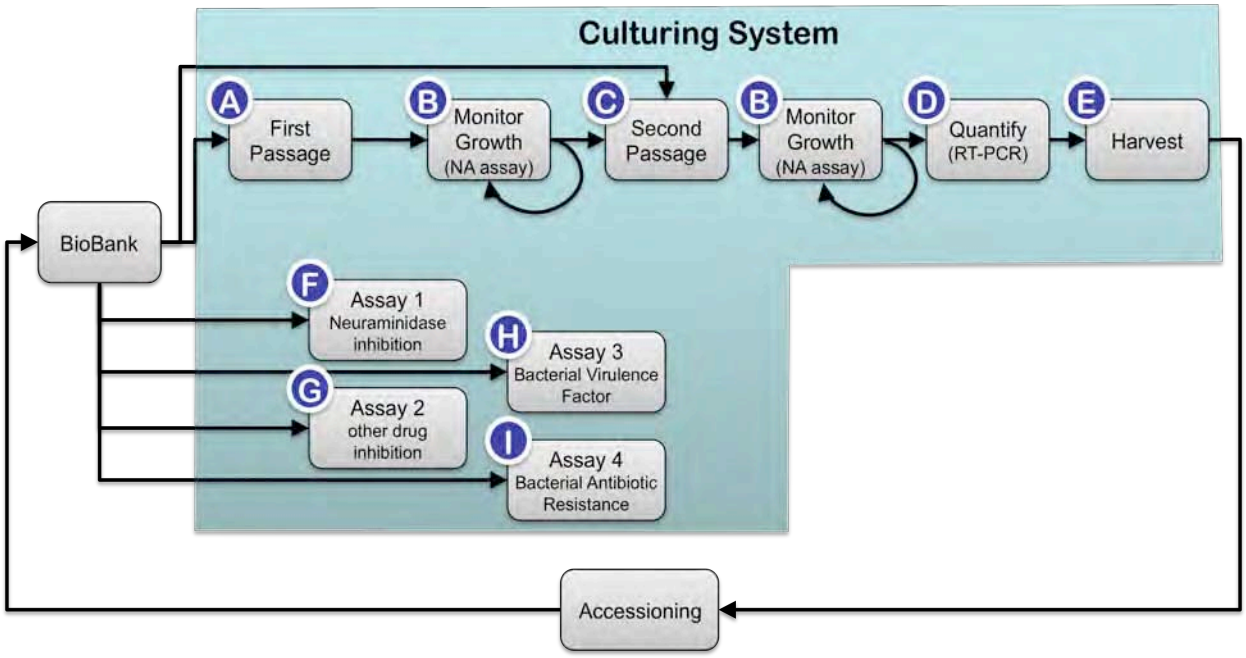


Figure 15. Overview of the Culturing System operations

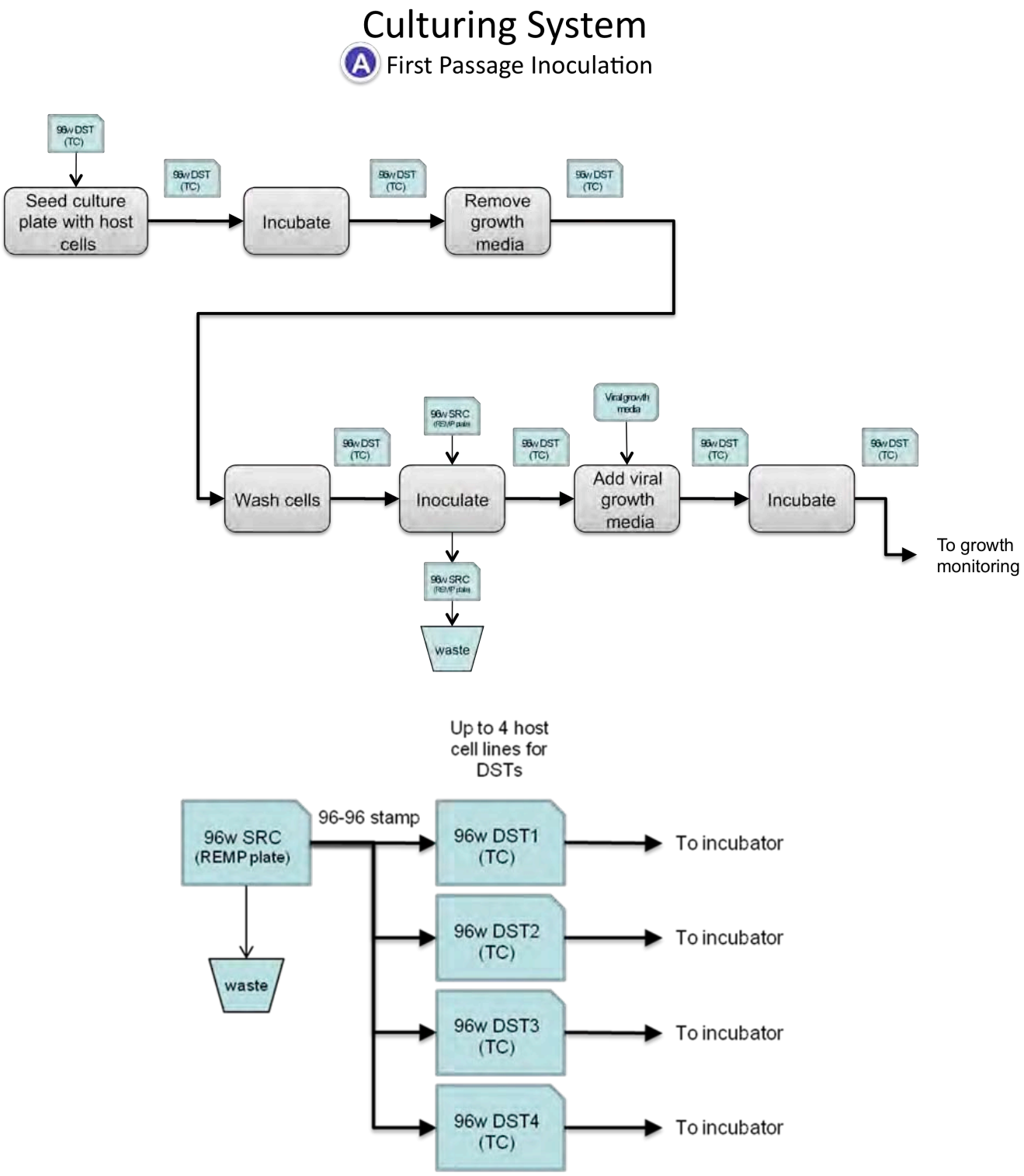
The supported protocols by the culturing system are:

A. First Passage Inoculation
B. Daily Growth Monitoring
C. Second Passage Inoculation
D. Viral Quantification
E. Viral Harvest

Additionally, several assays may be supported, including:

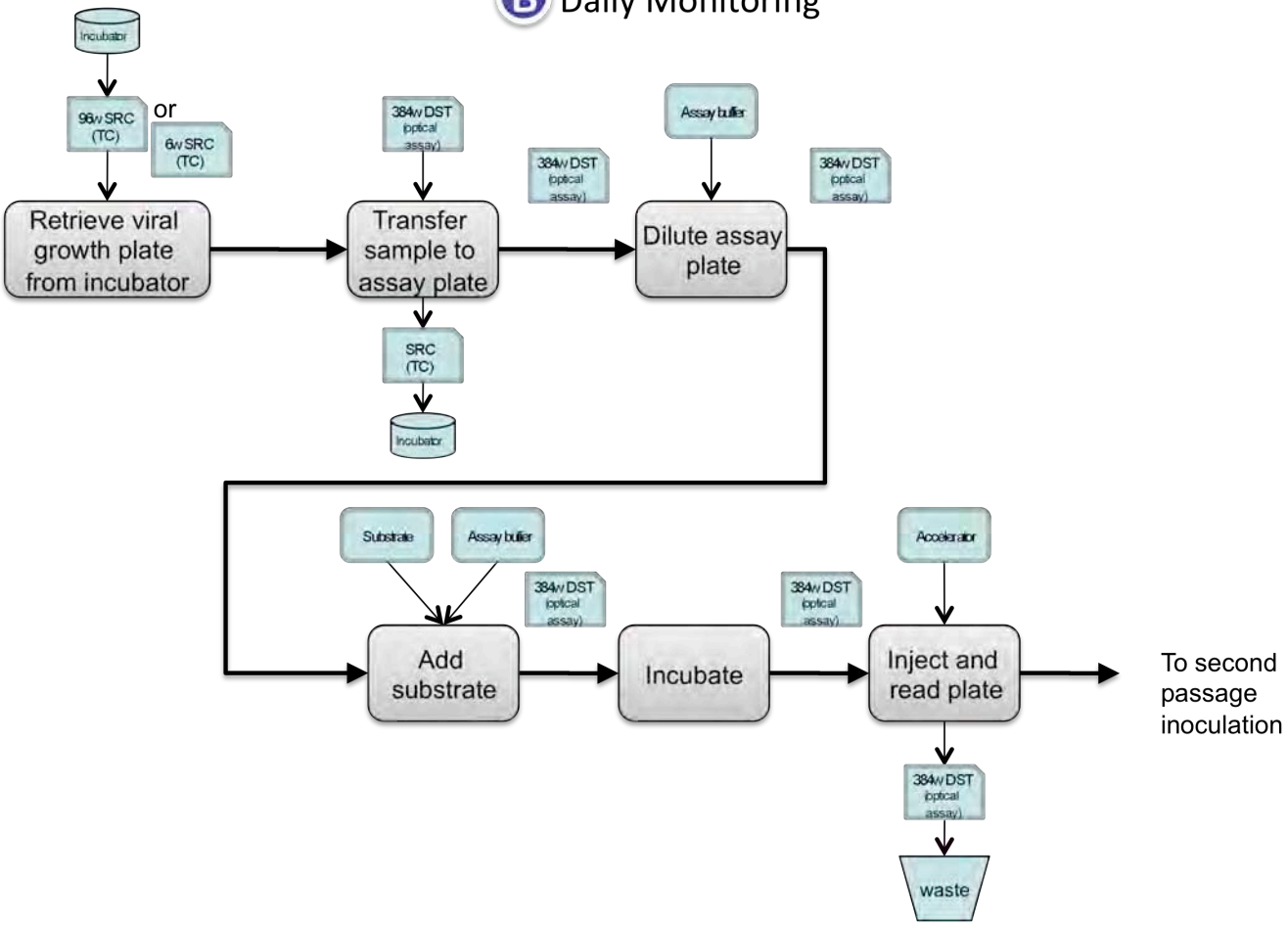
F. Neuraminidase Inhibition assay
G. Other Drug inhibition assay
H. Bacterial virulence factor assay
I. Bacterial antibiotic resistance assay

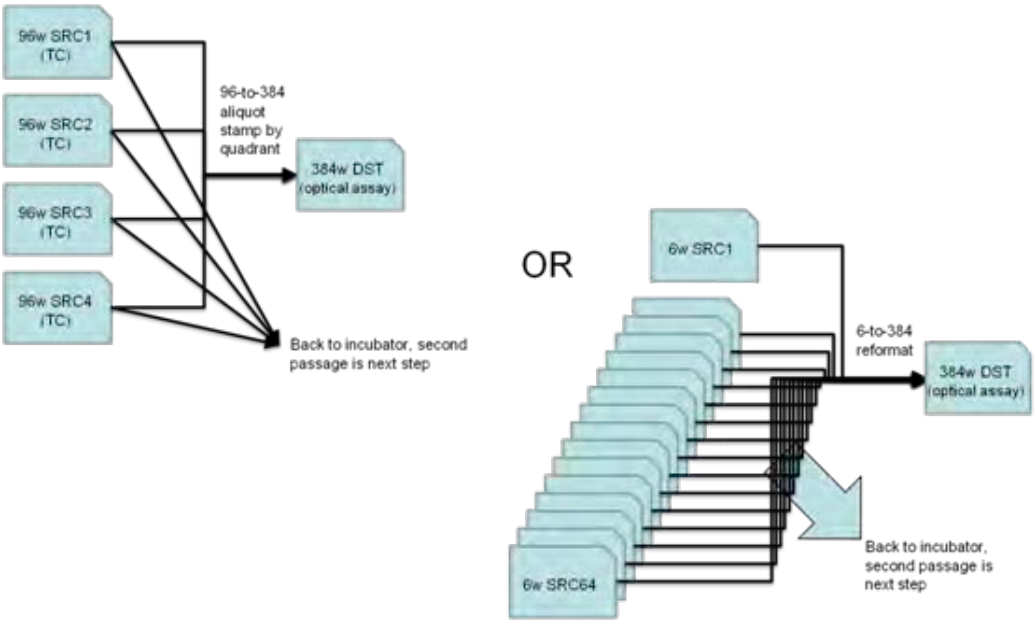
These protocols and their associated plate-to-plate mapping are depicted in detail in the following figures.


Culturing System
First Passage Inoculation
96w DST (TC)
Seed culture plate with host cells
Incubate
Remove growth media
Wash cells
96w SRC (REMP plate)
Inoculate
Viral growth media
Add viral growth media
Incubate
To growth monitoring
waste
Up to 4 host cell lines for DSTs
96w SRC (REMP plate)
96-96 stamp
96w DST1 (TC)
96w DST2 (TC)
96w DST3 (TC)
96w DST4 (TC)
To incubator
waste

Culturing System

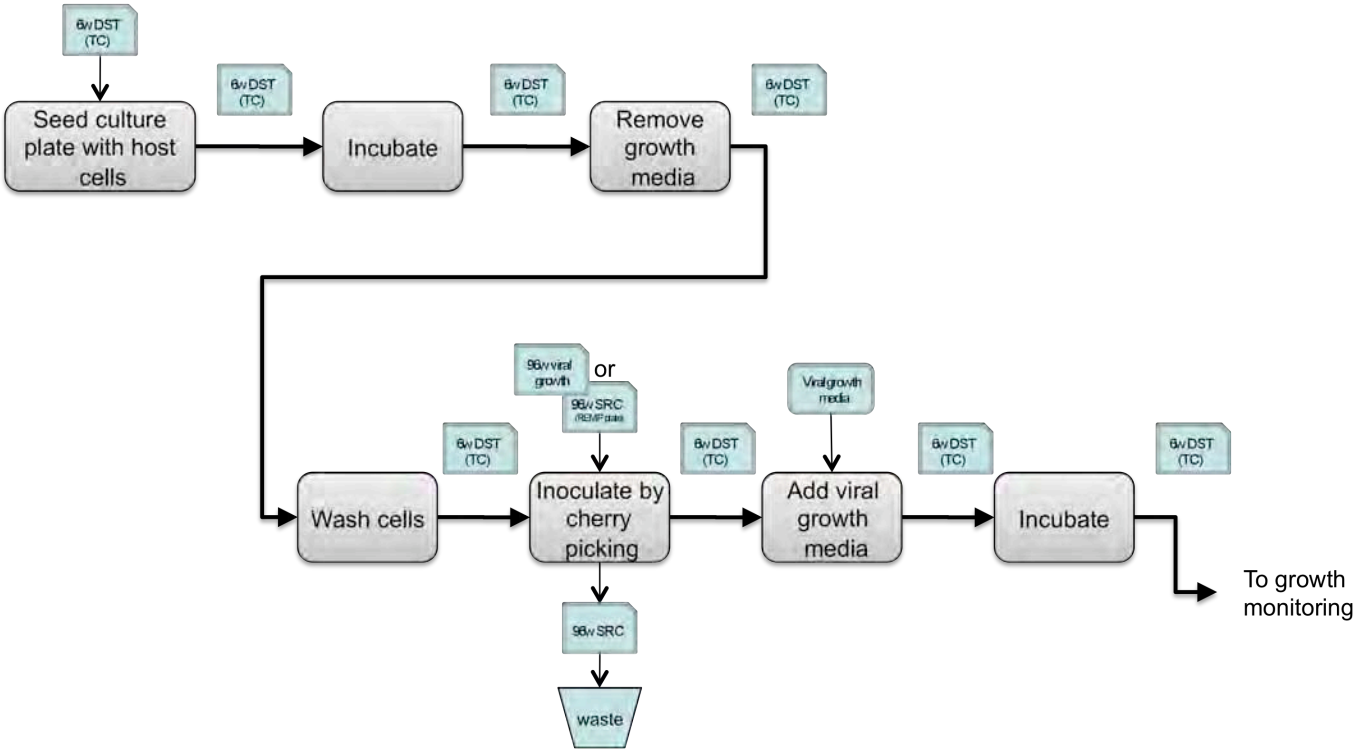
B Daily Monitoring

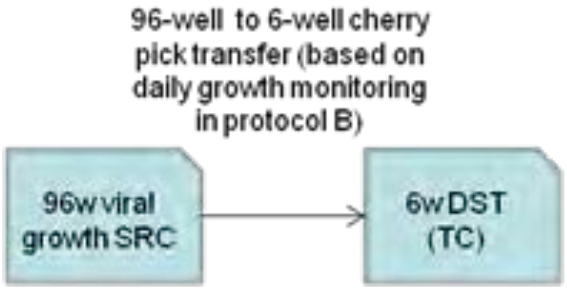




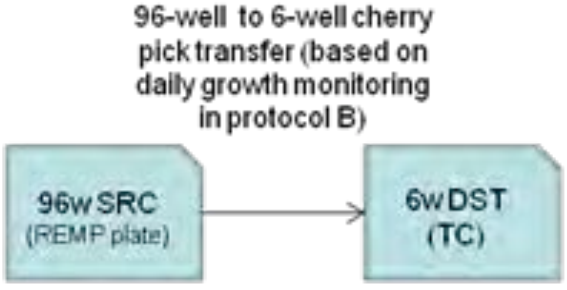
Culturing System

Second Passage



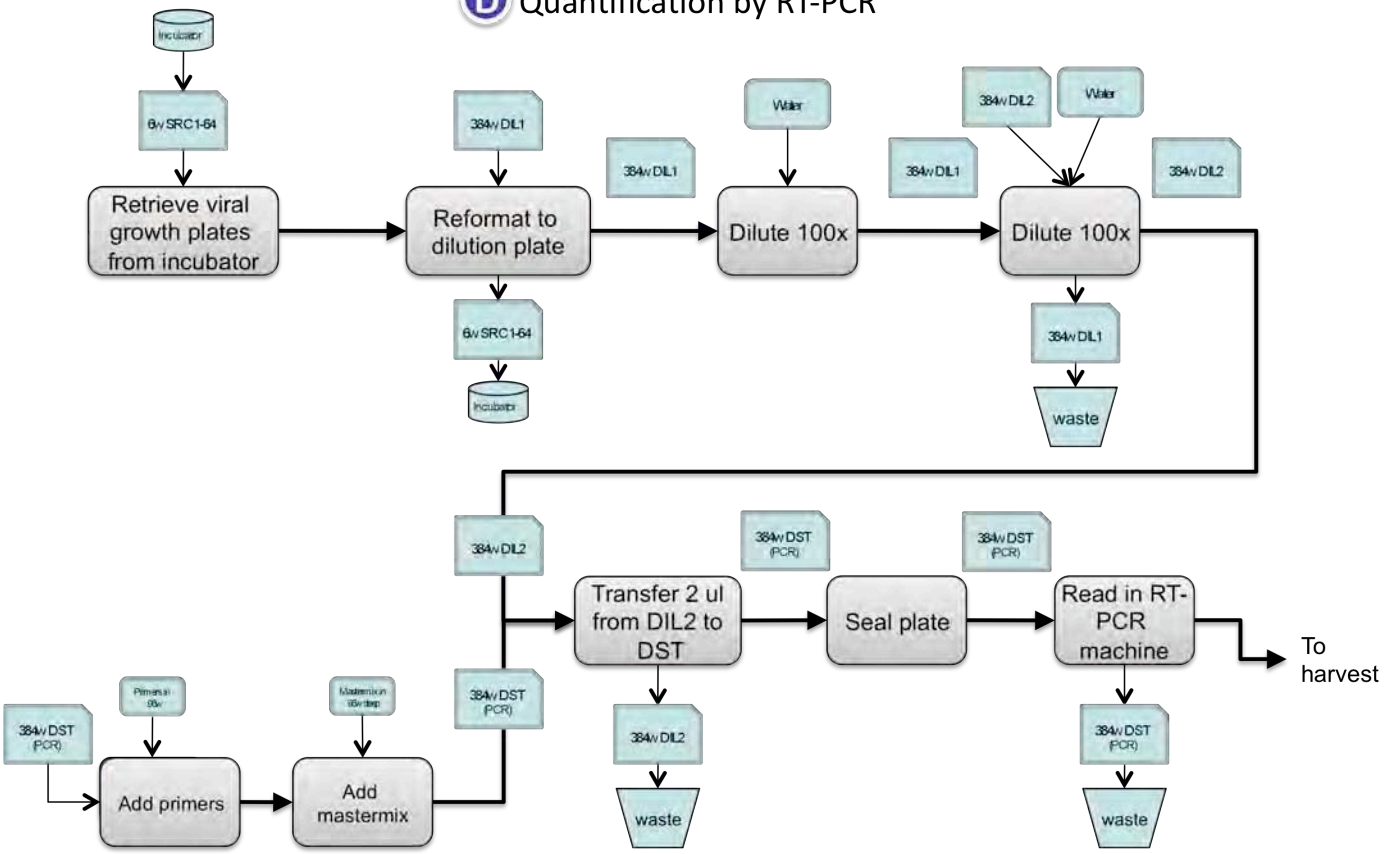


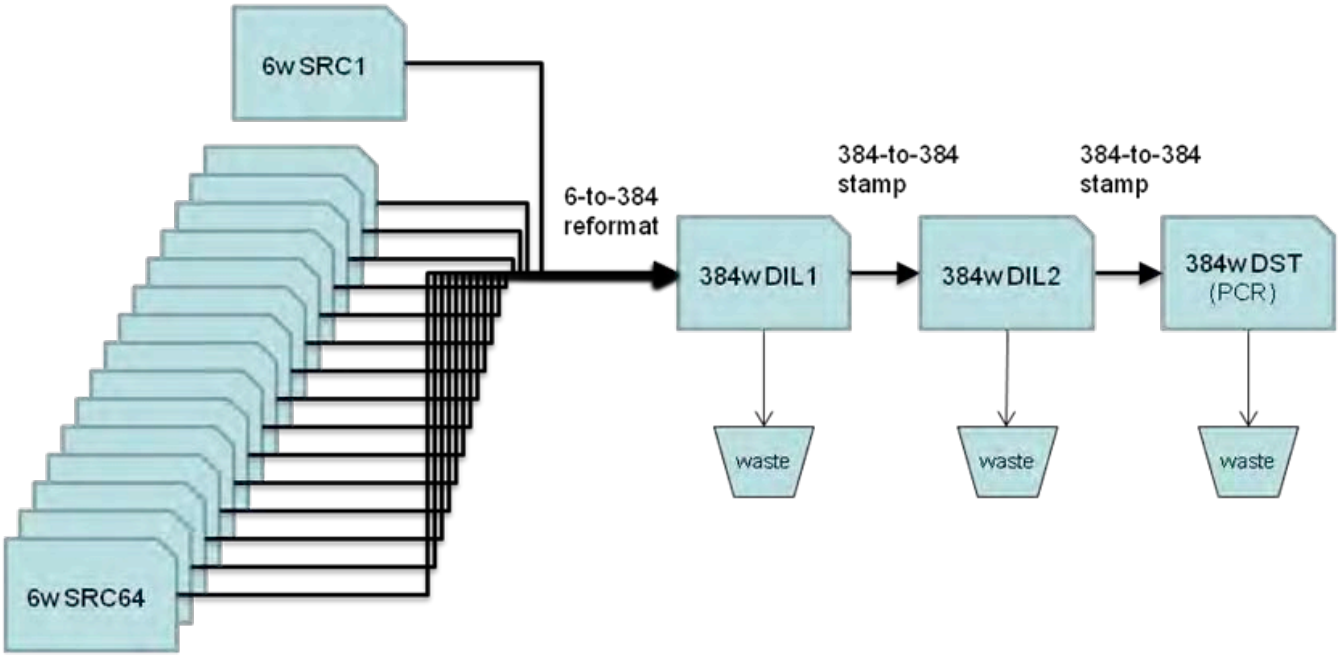
OR



Culturing System

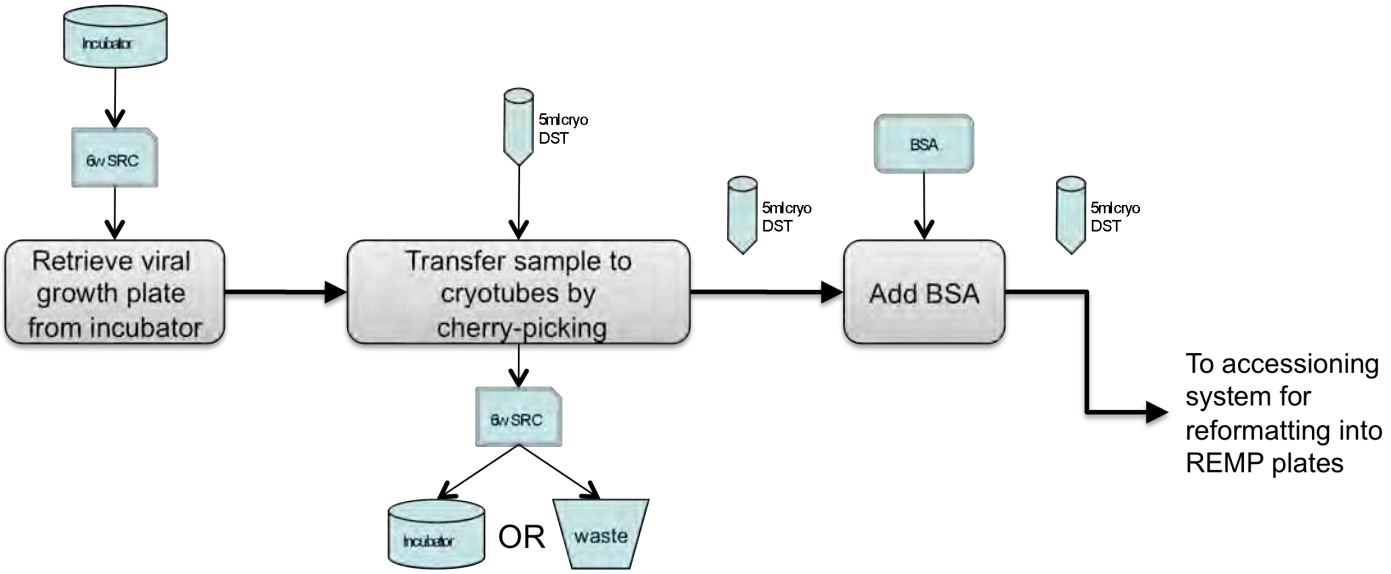
Quantification by RT-PCR

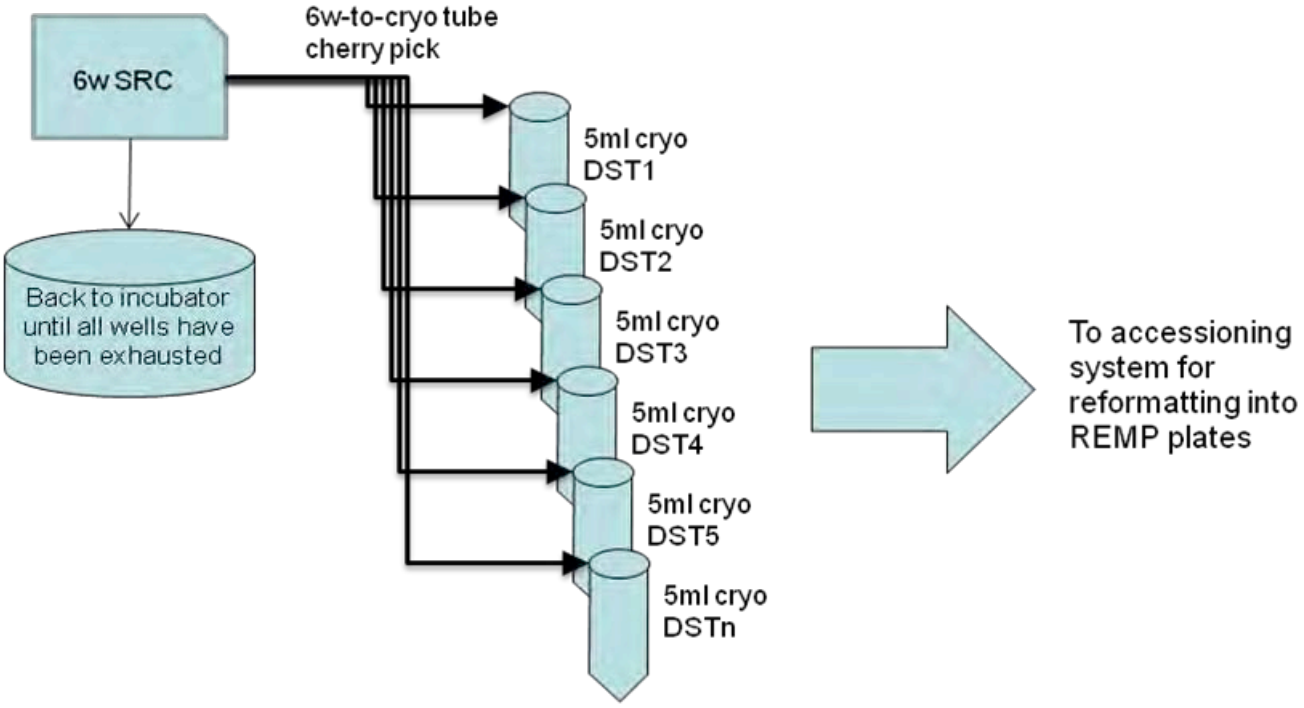




Culturing System

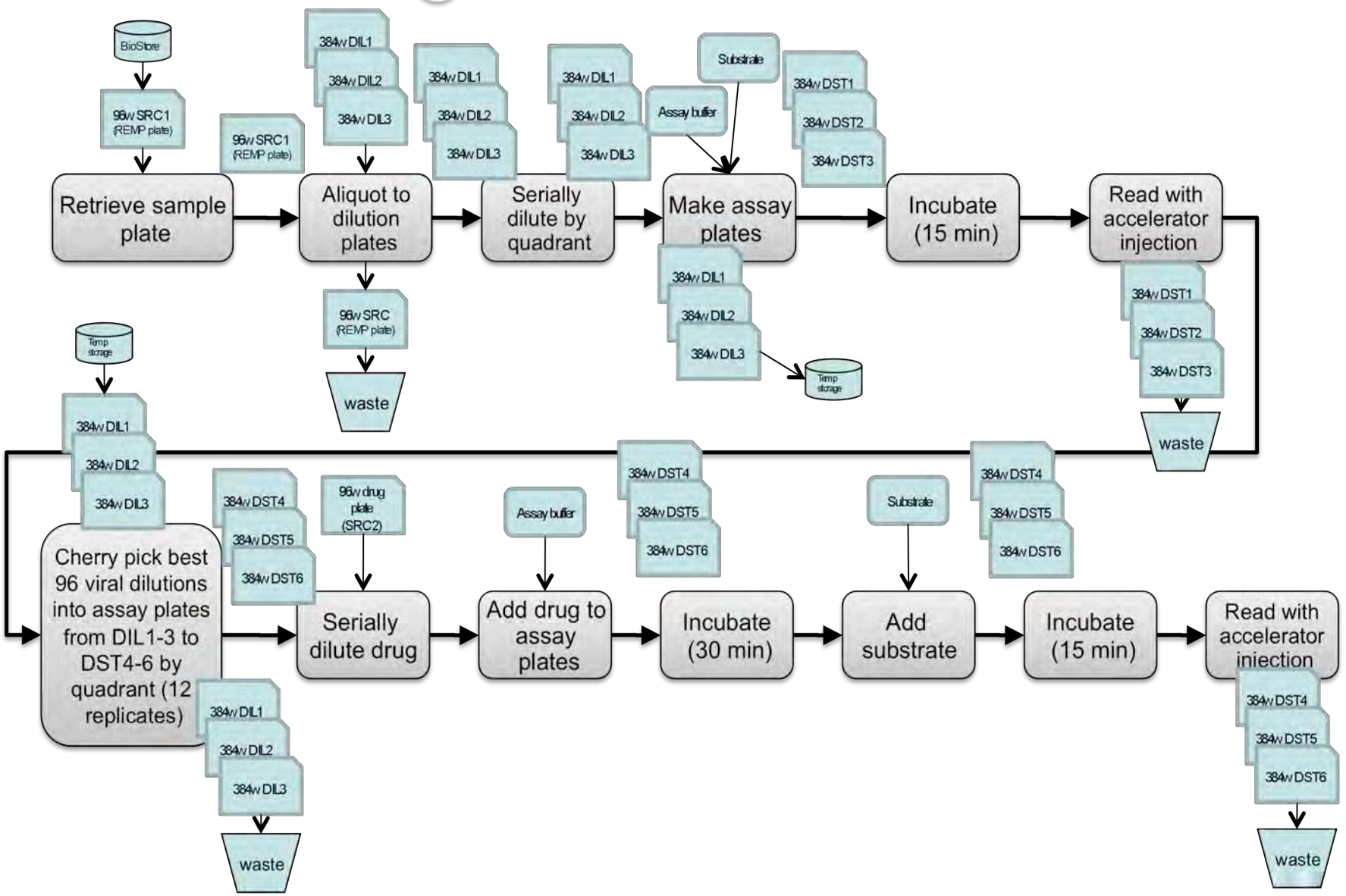
Harvest





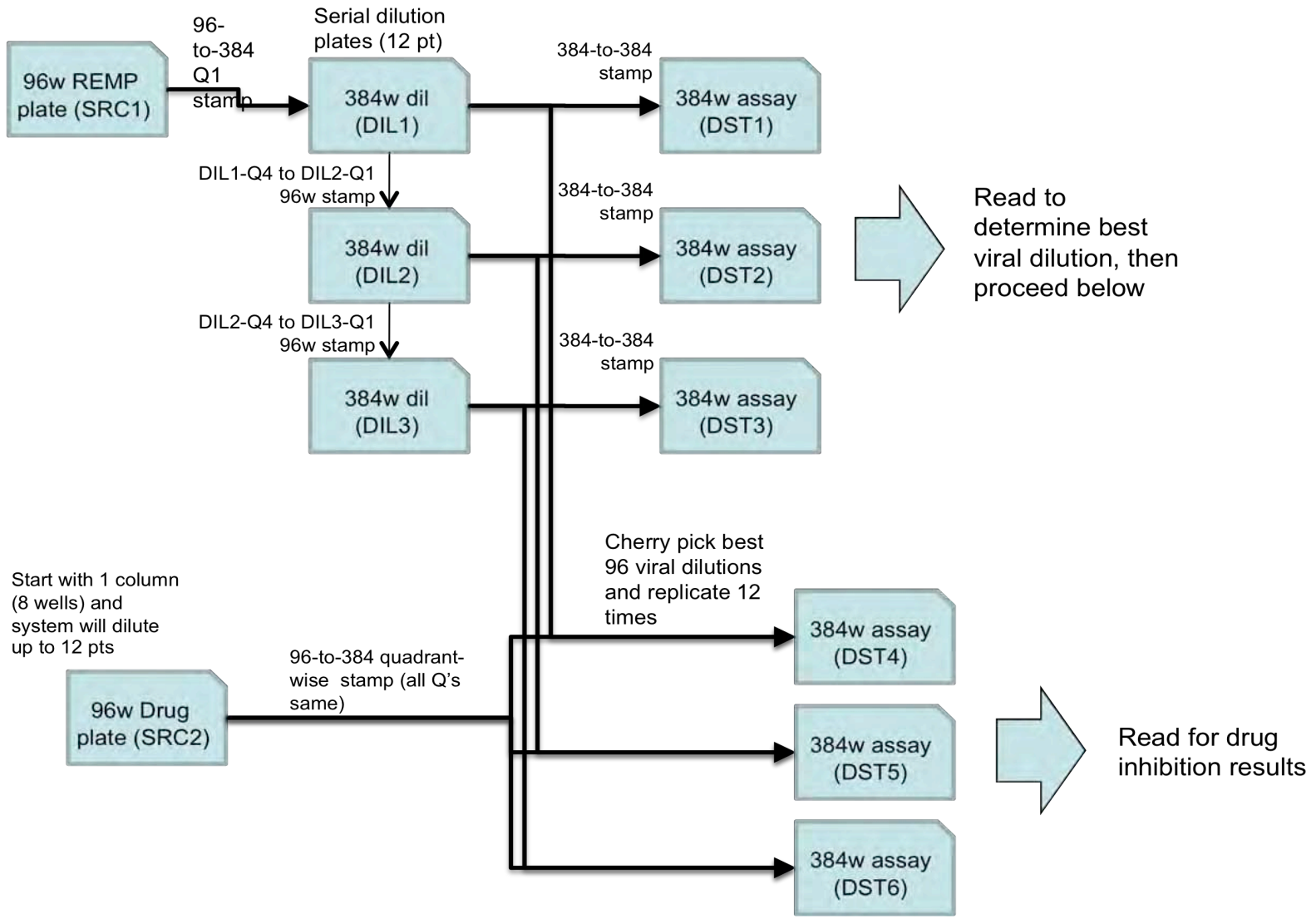
Culturing System

Neuraminidase Inhibition Assay



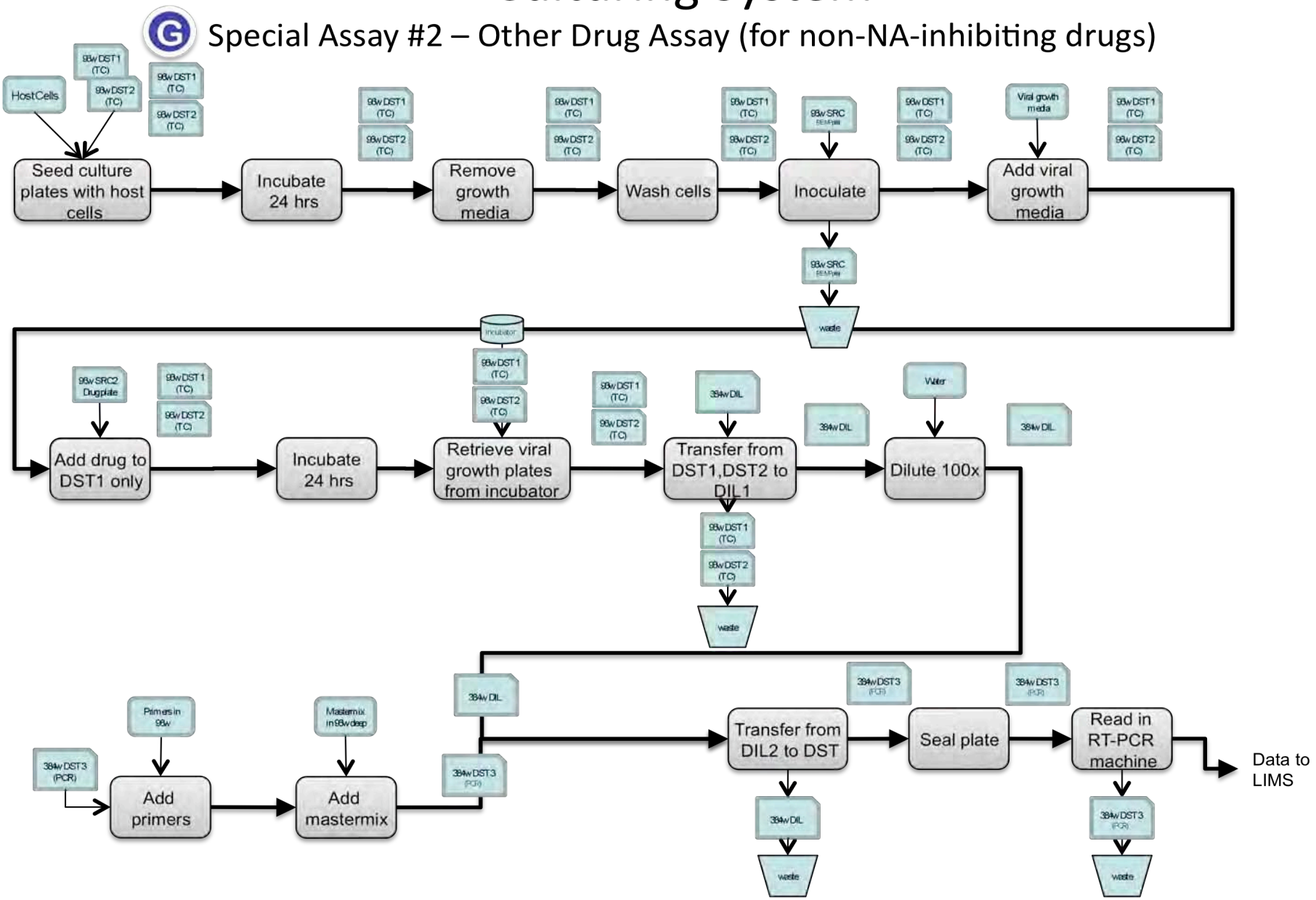
Culturing System

Neuraminidase Inhibition Assay



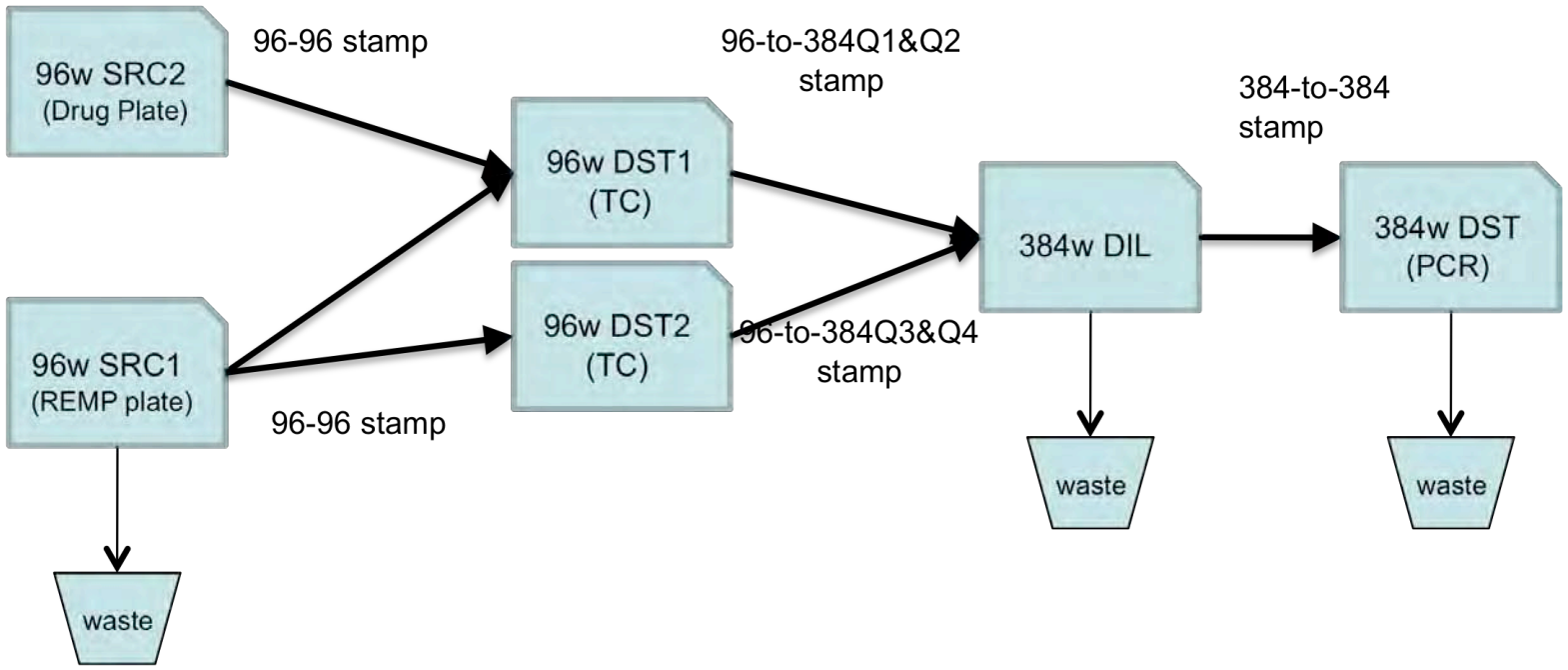
Culturing System

Special Assay #2 – Other Drug Assay (for non-NA-inhibiting drugs)



Culturing System

Special Assay #2 – Other Drug Assay (for non-NA-inhibiting drugs)



System Specifications

The system specifications for the RFQ are summarized below.

Purpose and Functionality Requirements

1. The first order requirement: establish a culturing system to amplify viruses (in mammalian cell lines) and bacteria using a fully automated system requiring minimal human input of labor or handling.
2. Integration with LIMS for workflow and data management
3. Capable of performing a variety of detection assays, including:
    a. Real time RT-PCR
    b. Culture growth and sterility monitoring
    c. Micro-neutralization phenotyping assays
    d. Cell-based assays
4. Flexible and compatible with public health methodologies and reagents

Operational Requirements

1. A fully automated system, with minimal human intervention
2. An integrated system, with components to accommodate the following tasks:
    a. Grow viruses on a mono-layer of mammalian cells, in micro-well plates, with multiple cell lines
    b. Grow and assay bacteria in user-defined assays
    c. Screen samples, and monitor growth and sterility at regular intervals
    d. Perform real time RT-PCR
    e. Antibody-, substrate-, and/or cell-based assays
3. Able to handle a large number of samples
    a. Hold 200-250 128 x 86 mm plates per incubator
    b. Have multiple incubators that can be readily sterilized and switched between human and animal samples
    c. Expect ~ 4 different cell lines (for influenza virus growth in particular)
    d. Anticipate surge capacity of 20,000 readouts daily during pandemic
4. Data transfer between Culture System software and Laboratory Information Management Systems such as StarLIMS using web services (see separate web services document).
5. Flexible construction, allowing for change and exchange of pieces of equipment when required and/or as technology evolves
6. Easy access to all integrated devices for service and maintenance
7. Minimize downstream costs of consumables, including tips, reagents
8. Able to withstand cleaning with 10% household bleach and vaporized hydrogen peroxide
9. System shall provide uninterruptible power for a minimum of 5 minutes.
10. In the event of power loss, system will finish ongoing operations and pause.

Safety Requirements

1. This system is expected to run with live pathogens on the deck. Personnel, product, and environmental protection needed. Enclosure with directional airflow and HEPA-filtered exhaust for the system will be required.
2. Emergency stops
3. Interlocked access doors to system enclosure
4. Clean to dirty workflow and airflow
5. Waste should be stored in such a manner that it does not pose a safety or aerosol hazard to personnel. Waste should also be stored so that it does not pose a contamination hazard to the product or workspace.
6. Access to refill reagents and consumables should be accomplished without breaching safety containment enclosure.
7. Optional fire suppression for the interior of the safety containment enclosure should be provided. Exercise of this option will be determined at purchase order placement.

Required System Components:

1. Incubators:
    a. Four at minimum (to separate human and animal influenza viruses)
    b. Two incubators should be capable of shaking for bacterial growth
    c. 200 - 250 plate capacity per incubator
    d. 35-37°C, 5%CO2
    e. Hold sealed or lidded micro-plates
    f. Self-cleaning
    g. Plates moving in and out of incubators daily for culture sampling and growth monitoring
    h. Robotic track or other mechanism to send the plates between incubators and liquid handler
    i. Daily harvesting from culture wells with satisfying viral titer
    j. Optional 5th incubator for cell line maintenance
2. Refrigeration:
    a. 4°C for media and cell culture components
    b. Accessible to liquid-handling platform
    c. Technician must be able to access/refill reagents without breaching safety containment enclosure.
3. Plate storage, handling:
    a. Hotel (for microplates, tips) capable of presenting tips if needed and presenting at least seven different microplates

b. Technician must be able to access/refill plates and consumables without breaching safety containment enclosure

c. Robotic arm for plate transfer

d. Should be able to handle REMP plates, microplates, and 5 ml cryotubes with pierceable tops or screw-caps

4. Liquid handling:

a. Media components from refrigeration unit to multiwell-plates and roboflasks

b. Handle virus inoculation: Remove media, wash, and move viral sample from REMP tube to multi-well plate for growth (including foil piercing of tubes)

c. Move small volumes (<5 µl) from growth plate to assay plates

d. Move entire volume (100 µl – 3 ml) from growth plate to cryotube for biobanking

e. Sampling at certain time intervals

f. Cherry picking, serial dilutions, and variable span/interwoven dispensing for 6-, 96-, and 384-well plates

g. Cherry picking and reformatting should be able to accommodate 5 ml cryotubes with either pierceable tops or screw caps

h. 96-well to 96-well and 96-well to 384-well liquid transfer stamping capability

i. Culture reagents will need to be warmed to 35-37ºC before distribution

j. Fixed tips with washing/sterilization or disposable tips to minimize cross contamination

k. Multidrop (or equivalent) with stir plate to withdraw freshly prepared cells and cell culture media (technician provides trypsinized cells

5. Detection Assays:

a. Plate reader with injection/dispense capability:

- Fluorescent
  - excitation spectra 360/40, 485/20 530/25
  - emission spectra 460/20, 528/20, 620/40, 645/40
- Luminescent (top-reading)
- UV/Vis λ range 200-999nm
- Capable of reading and injecting into 6-, 12-, 24-, 48-, 96-, and 384-well plates

b. Cell culture quality control can be done on plate reader or Real-time

c. Real-Time PCR Machine capable of handling 384 samples at one time

- Machine should be able to do both fast and slow cycling.
- To prevent contamination of samples with PCR product, Real-Time PCR should not be in the same compartment with incubators or liquid handler

6. Optical membrane sealing, Plate-lidder/delidder(s):

a. Multiwell-plates growing cells/virus covered with plate lid

b. Plate Sealing/peeling/lidding:

- Plate sealing and peeling may produce unwanted aerosols that could cross-contaminate samples. In addition, repeated plate sealing and peeling may leave

gummy residue on plates that prevents a good seal from being produced. Lidding may be a slow process and produce edge effects. No solution is ideal. In the documents below we describe the plate as having a lid. We will consider manufacturer's recommendations for a solution to this issue as well as manufacturer's tests for cross-contamination using sealers/peelers/lidders

c. Real-time plates covered with optical film

d. Assay plates to plate reader need to have cover removed before reading (for reads with injection), or have the same optical film as Real-time plates

7. Barcode readers

   a. Barcode reader for micropolates

   b. Barcode reader for 5ml cryotubes

Other Details in Technical Requirements:

1. Input of 0.30 ml REMP tubes from accessioning/biobanking system
2. Output of 5 ml cryo-tubes to accessioning system with pierceable tops or screw caps.
3. Culture Waste will be at BSL2 level and possibly BSL3. Solid and liquid waste should be able to be easily transported by a technician. Technician will autoclave solid waste and will add bleach (10% final concentration) to liquid waste before disposal.
   a. All solid waste should be automatically bagged for safe transport to autoclave.

Expected Reagent and Disposable Materials to be Used:

1. Cell lines (MDCK, chicken embryo fibroblast, others)
2. Media (generally DMEM without phenol red, high glucose – media requirement could change as we try more cell lines)
3. Antibiotics/Antimycotics
4. TPCK-treated trypsin
5. Pipette tips (or sterilized/washed tips if can be shown to completely prevent cross-contamination
6. Microplates: cell-culture-treated, 6-, 48-, and 96-well
7. Optical Microplates: white 384-well
8. Optical PCR plates: 384-well
9. Optical film on a roll
10. Real-time one step master mix
11. Real-Time Probes and Primers
12. Controls such as, real-time standards
13. NA-STAR and buffers
14. PBS
15. BSA, fraction V
16. zanamivir and oseltamivir, amantadine, rimantadine

17. Waste bags

Sterility Monitoring:

We will consider manufacturer's recommendations for sterility monitoring of samples.

1. Samples are from human and animal sources, and may have unwanted contaminants such as fungus or bacteria. We plan to monitor viral samples using 16S RNA
2. Cross-contamination between samples is an undesirable possibility and must be minimized.

Throughput Estimate:

1. Maximum of 500 new samples input per day
2. Incubation: 220 plate incubators = ~1300 to ~21,000 samples per incubator (depending on storage plate type)
3. Up to ~5,000 real-time PCR reactions per day (in full surge capacity)

Dimension Requirements:

1. Footprint of assembled system should be no larger than 12' x 8' with overall height less than 7 feet
2. 4 foot access buffer around system
3. Disassembled components should be no bigger than 44" x 84"

Possible Vendor solutions for the Culturing system are shown below:

Figure 16. Matrical MACCS example system

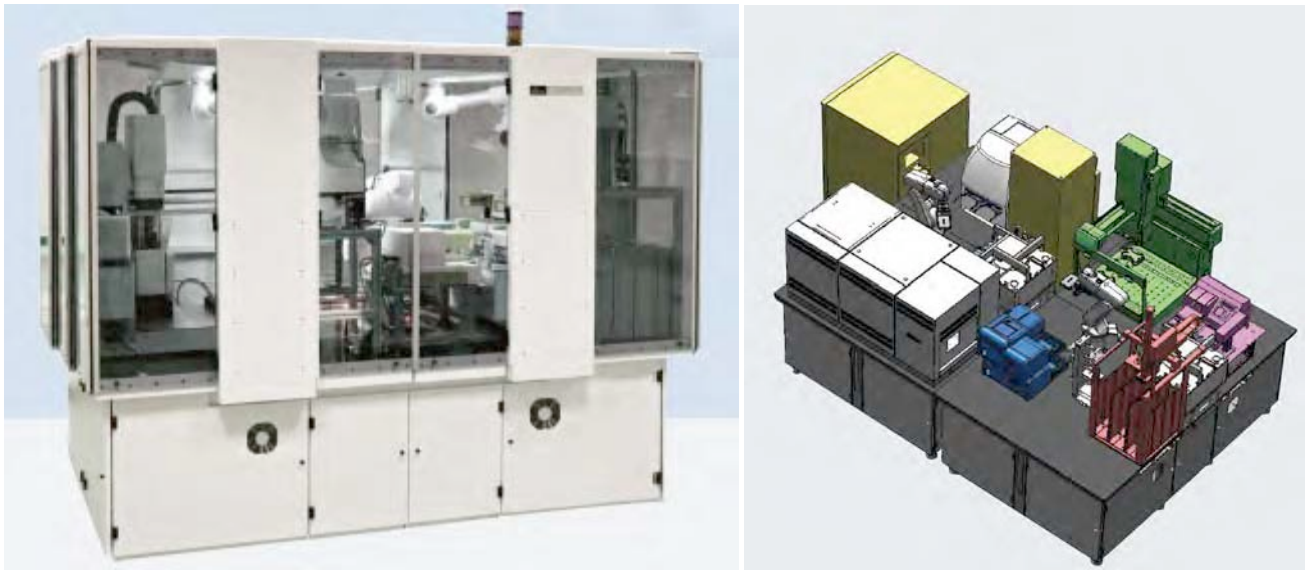


Figure 17. Perkin Elmer cell::explorer example system

Figure 18. The Automation Partnership Cello example system

Figure 19. Agilent/Velocity 11 BioCel example system

Figure 20. Beckman example system

Figure 21. HighRes Biosolutions example system

Concerns

1. The ultimate output of the culturing system as specified is in 5ml cryo tubes. These tubes must be fed back through the Accessioning system for reformatting into REMP storage plates for storage in the Biobank. Perhaps a more efficient design would aliquot the cultured samples directly into REMP plates, thus bypassing the already bottlenecked Accessioning system. One criticism of this suggestion is as follows: as the REMP plates need to be sealed and tubes individually cut/separated, and a sealer/cutter is financially expensive and already exists on Accessioning system, providing direct-to-REMP in the culturing system may not be financially feasible. However, since the REMP biobank is no longer in the works, perhaps switching away from the REMP storage plates to a more economical and compatible solution should be considered. Matrical, Micronic, and Matrix all supply similar tube racks.

2. A second concern is how the negative controls on the daily growth monitoring plates will be implemented. At least one well of each assay plate needs to have a negative control (presumably a PBS-only well). This could be accomplished by
   - Biobank inserting a tube of PBS into say A1 of every input plate, or the first of every 4 inputs plates (since four 96-well plates will be stamped onto one 384-well assay plate)
   - Biobank inserting an empty tube into A1 of every input plate, or the first of every 4 inputs plates (since four 96-well plates will be stamped onto one 384-well assay plate). Then downstream (like here in the culturing system), PBS would be added to this well(s)

- Human loading either an empty tube or a PBS tube into A1 of each culturing REMP plate before loading into Biobank. This way the Biobank would only punch 95 samples into each culture REMP plate.

3. The expansion in scope of this system to include bacteria is worrysome. Co-mingling of mammalian cells and bacteria within the same system (even if not concurrently) is fraught with trouble. This system is fairly complex with mammalian alone, and expansion of scope here may jeopardize its successful implementation.

Phenotyping System

The Automated Phenotyping System is an integral component of the overall automation design in that it will provide phenotypic data of the type that is traditionally used for typing strains of influenza, which can then be correlated with detailed genetic data (obtained from the Automated Genotyping System) and epidemiological data (obtained as meta data when samples are collected). The Automated Phenotyping System will be used to perform Hemagglutinin Inhibition (HI) and other immunotyping assays on influenza viruses. HI assays will utilize reagents and protocols developed by the Center for Disease Control and Prevention (CDC) and World Health Organization (WHO) for vaccine strain selection activities.

Work on a detailed description of the specifications required for the Automated Phenotyping System has not yet been initiated.

Genotyping System

The Genotyping system of the HTLN performs the sample preparation for sequencing of samples. The input is a 96-well PCR plate with RNA samples ready for amplification by PCR. The system performs the sample preparation in four steps:

A. PCR setup
B. PCR cleanup
C. Sequencing setup
D. Sequencing cleanup.

An overview of the process is shown in Figure 22.

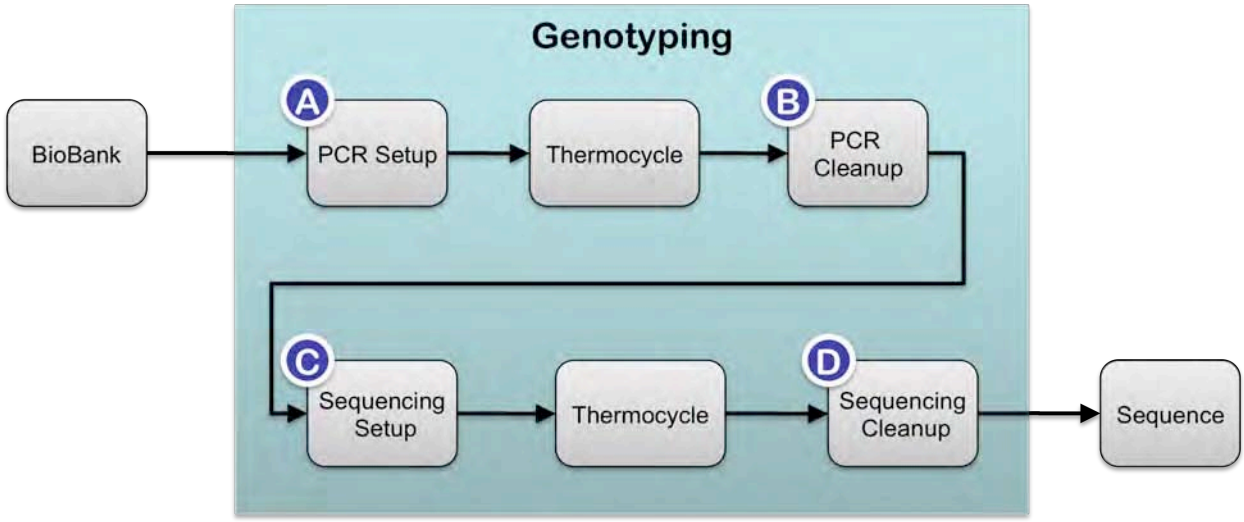
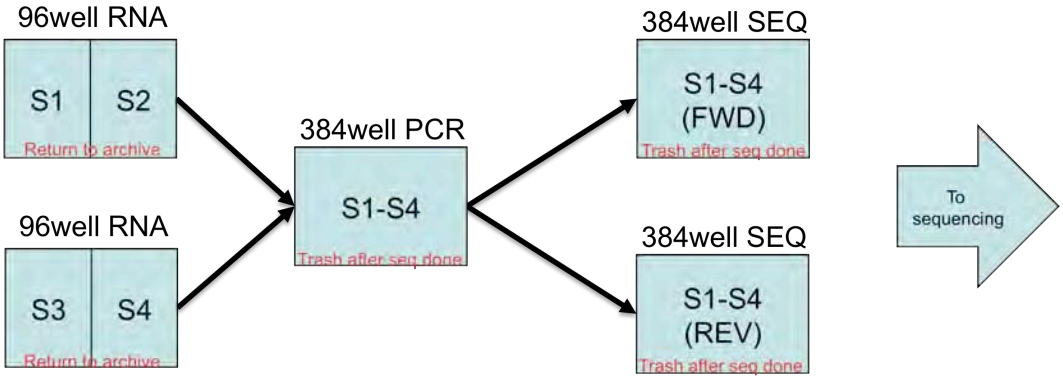


Figure 22. Overview of the Genotyping System operations

The system has been developed to be flexible in accommodating a variety of primer sets. As an example scenario, Figure 23 shows an overview of the process when set of 96 primers are used. All PCR and Sequencing reactions are performed in 384-well plates to minimize reagent use, and therefore costs. In this scenario, four RNA samples are input to the system on 2 96-well plates. These samples are "stamped" into a 384-well PCR plate by quadrant, thermocyled, and then "stamped" into two 384-well SEQ plates for eventual sequencing. In this case, 40 96-well RNA plates containing 20 unique RNA samples are processed in 12 hours with the 96 primer set.

Genotyping Overview

Scenario: 96 primer set



- Each 96well RNA plate contains 2 samples in 48 wells each (6 cols each)
- Each 384well PCR plate has 4 RNA samples (by quadrant)
- Each 384well SEQ plate has 4 RNA samples (by quadrant), either FWD or REV (by plate)

Each 12 hour run:

- 80 RNA samples (40 96well RNA plates)
- 20 PCR plates (384well plates)
- 40 sequencing plates (384well plates)

Figure 23. Overview of Genotyping process, showing a 96-primer scenario

The system is currently undergoing system validation and system integration at LANL. Figure 24 shows a "cartoon" layout of the system on the left, and a photo of the system in action on the right.

Protocols

The following four figures depict the details of the four steps of the Genotyping system.

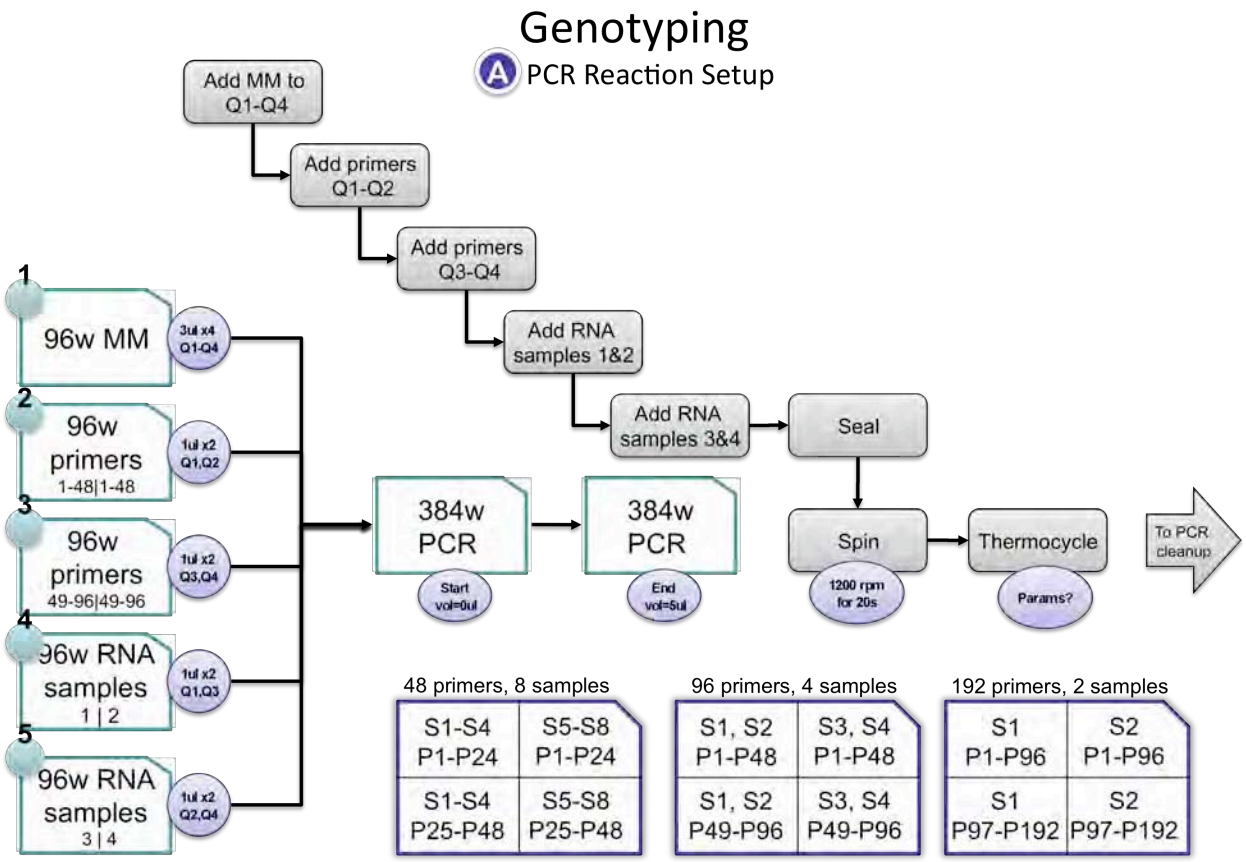


Figure 25. PCR setup step. RT-PCR and PCR are carried out in a one step process using Qiagen's OneStep RT-PCR kit. One microliter of RNA and 1ul total of forward and reverse primers are added to 3ul of master mix in a 384 well plate. The plate is sealed, spun down, and put into a thermal cycler for amplification. Lower three frames depict three possible scenarios of plate layouts with 48, 96 or 192 primers.

Genotyping

B PCR Cleanup

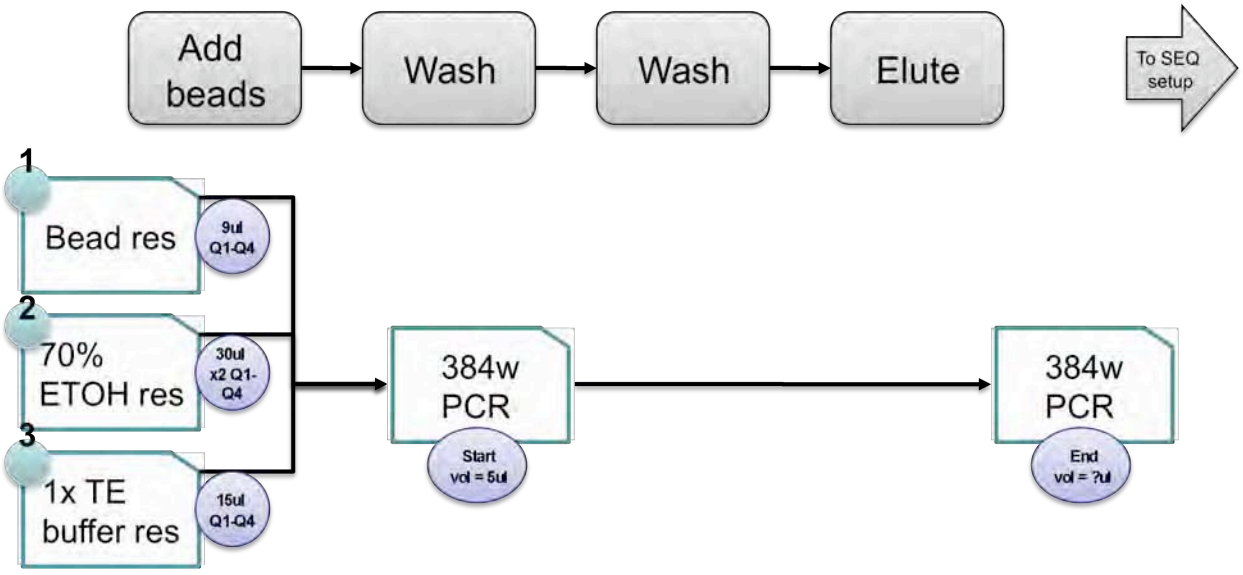


Figure 26. PCR cleanup step. PCR products are cleaned using Agencourt's Ampure bead based technique. The 5ul reactions are cleaned with 9ul of beads, washed 2 times with 30ul of 75% ethanol and eluted in 15ul of TE buffer and then plate sealed.

Genotyping

Sequencing Setup

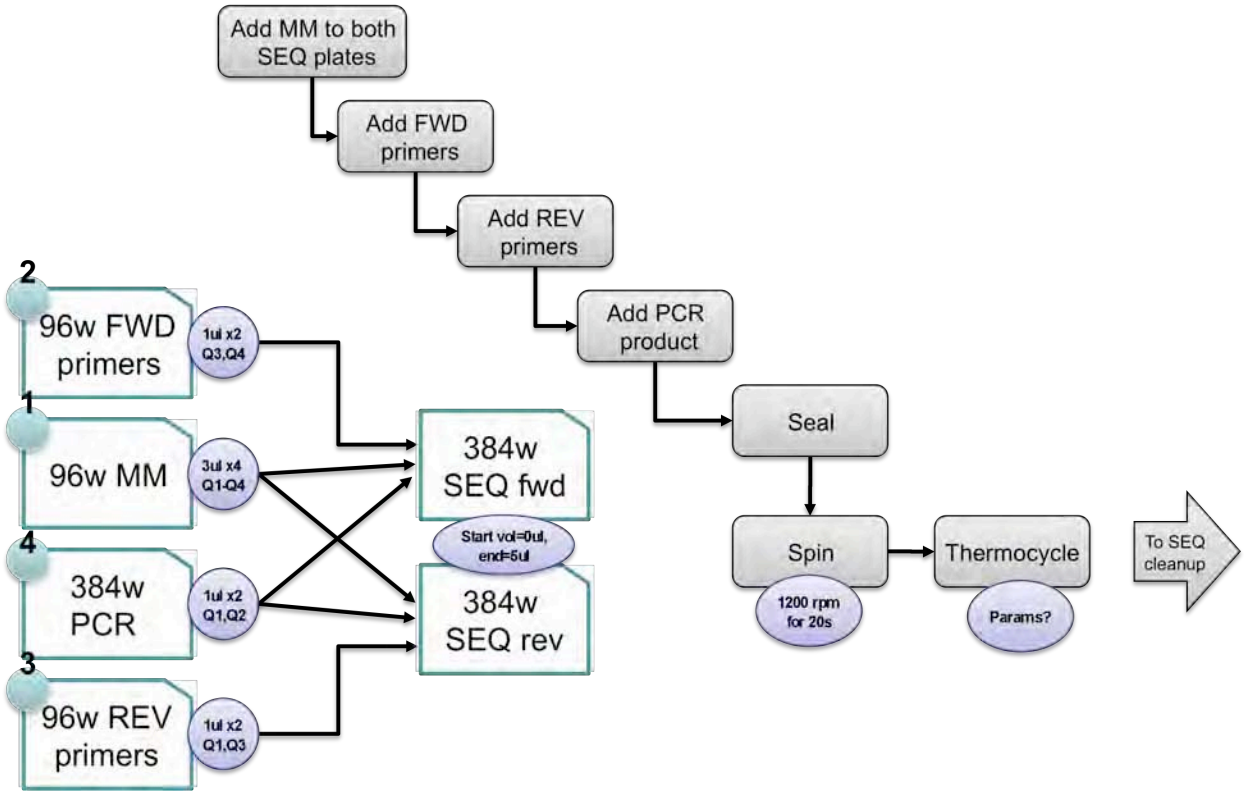


Figure 27. Sequencing reaction setup step. One microliter of cleaned PCR products are sequenced in 5ul reactions in a 384 well plate using ABI's Big Dye Terminator kit. M13 forward and reverse primers are used in the sequencing reaction. The plate is sealed, spun down, and put into a thermal cycler for processing.

Genotyping

D SEQ Cleanup

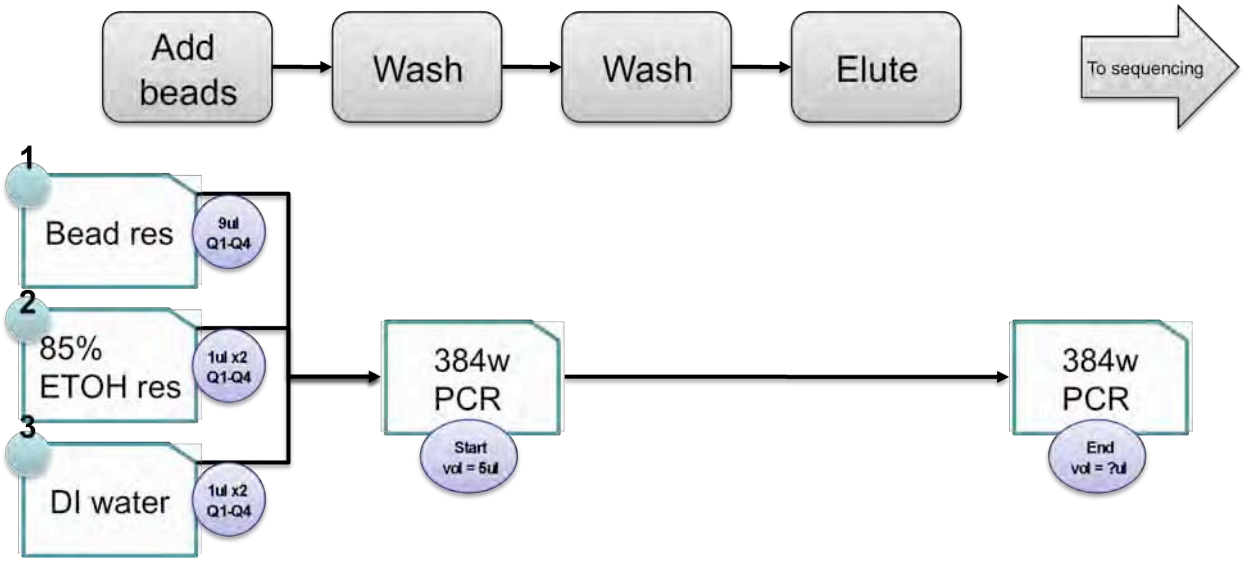


Figure 28. Sequencing reaction cleanup step. SEQ products are cleaned using Agencourt's Ampure bead based technique. The 5ul reactions are cleaned with 5ul of beads, washed 2 times with 14.3ul of 85% ethanol and eluted in 15ul of DI water and then sealed.

Concerns

1. The system solid waste disposal is causing problems with stray used pipette tips "spraying" up and out of the waste receptacle and causing physical obstructions leading to system errors. This might be remedied by:
    a. Modifying the configuration of the waste chute to be a ramp
    b. Adding a door-like mechanism on the waste chute opening to allow for one-way movement of objects

2. Tip wash water needs filters on inputs to prevent tip wash chimney clogs

3. Magnet beds on VPrep pipettors need to have active plate locking/capturing mechanisms to prevent pierced plates from sticking to pipette heads and causing system crashes

4. Overall system validation is not complete. The sequencing cleanup step does not appear to be working (yielding bad sequence data).

Viral Data Analysis

Our approach to the analysis and understanding of influenza (i.e., viral pathomics) involves both hypothesis-driven and data-driven science. To begin our work, we have established close collaborations with Dr. Nancy Cox, who is the Director of the Influenza Division at the Centers for Disease Control and Prevention (CDC) and also serves on our Scientific Advisory Board. The CDC has provided 600 influenza B samples and a qualified 96 pair primer set to whole genome sequence these influenza B viruses with the automated genotyping system that is currently situated at LANL. The influenza B samples were chosen to represent strains that have circulated worldwide over the past 10 years. The sequencing of the 600 influenza B viruses will be used to qualify and validate the automated genotyping system, prior to relocating it to the UCLA BSL3e facility.

To date, approximately 200 whole influenza B genomes have been sequenced, deposited into public databases, and analyzed by previous research efforts (Chen 2008). These sequences are primarily limited to temperate regions (primarily North America) that experience seasonal influenza epidemics. The sequencing of 600 additional whole influenza B genomes by our proposed efforts and from locations that include the tropics, where influenza circulates throughout the year, will be an important addition to information on influenza B.

Unlike influenza A viruses, which infect and circulate in many different animal species, influenza B viruses infect and circulate in humans only (Nobusawa 2006). In addition, influenza B viruses have diverged into two lineages that are commonly termed "B/Victoria" and "B/Yamagata." These two lineages are immunologically distinct and vaccination with either lineage induces only weak protection against the other. It has become somewhat problematic to predict which lineage will dominate and circulate in any given year, which represents a challenge to vaccine strain selection efforts. Our hypothesis-driven and data-driven approach to influenza B analysis focuses on improving our understanding of influenza B evolution and improving vaccine strain selection efforts.

We make the following hypotheses:

1. The envelope proteins (hemagglutinin and neuraminidase) and internal proteins of influenza B are driven by different evolutionary pressures and dynamics. The envelope genes evolve (drift) according to immune pressure (selective advantage for novelty). The evolution of internal genes is driven by a need to be compatible with the envelope genes.
2. The periodic reassortment of the internal genes of influenza B enables new external genes to appear and circulate.
3. To continue to evolve and avoid evolutionary dead-ends, influenza B must periodically reassort its internal genes.
4. There is a latent pool of novel internal genes, feasibly in the tropics, that enables influenza B to periodically develop novel reassortant viruses. This implies that influenza B viruses are not well mixed on a global (geospatial and temporal) scale.

To test and refine these hypotheses, we have begun data-driven analysis of the available whole influenza B genomes. This preliminary work is being used to gain insight into existing information and better prepare for our upcoming analyses of the additional 600 whole influenza B genomes that will be generated by LANL. One principal computational algorithm that we have used for this work is based on Bayesian Markov Chain Monte

Carlo (MCMC) methods and statistical models (BEAST) that yield information such as phylogeny (trees), mutation rates (for branches and gene segments), lineage divergence (most common ancestors). These methods enable us to analyze influenza reassortment patterns (continuous, punctuated or a combination of both), the co-evolution of segments and the internal dynamics of new reassortants, and to learn from success and failure of such reassortants (Drummond 2005). The other principal computational algorithm that we have used for this work is based on Pseudo Maximum Likelihood Methods (PMLM) that yield phylogeny (trees), genome space analyses, amino acid space analyses, and conservation/covariation information (Bruno 1996). Data-driven examinations that we will undertake pertain to: 1) segment covariation in amino acid space versus nucleotide space; 2) direct comparison of what is learned from MCMC versus PMLM methods; and 3) if findings from the two methods are the same or different, then why is this the case and what is implied and/or learned from these similarities and differences.

In the next phase, our major and practical goals are to enable better predictive methods for selecting influenza B vaccine strains based on whole genome analyses, and to identify and/or predict potential new strains that may become dominant in coming influenza seasons. With the addition of phenotypic information on hemagglutinin and neuraminidase, we will also seek to enhance the power of the above methods. In addition, the use of other metadata (such as geospatial and temporal information and host characteristics) will enable the development of multi-scale epidemiologic models that bridge genomic, proteomic and population data in space and time. Such models will allow better prediction of influenza B evolution, spread, and effects on diverse host populations.

HTLN LIMS

The HTLN Laboratory Information Management System (LIMS) provides a information management and system control for the laboratory. The LIMS is connected to each system via a webservices interface connection.

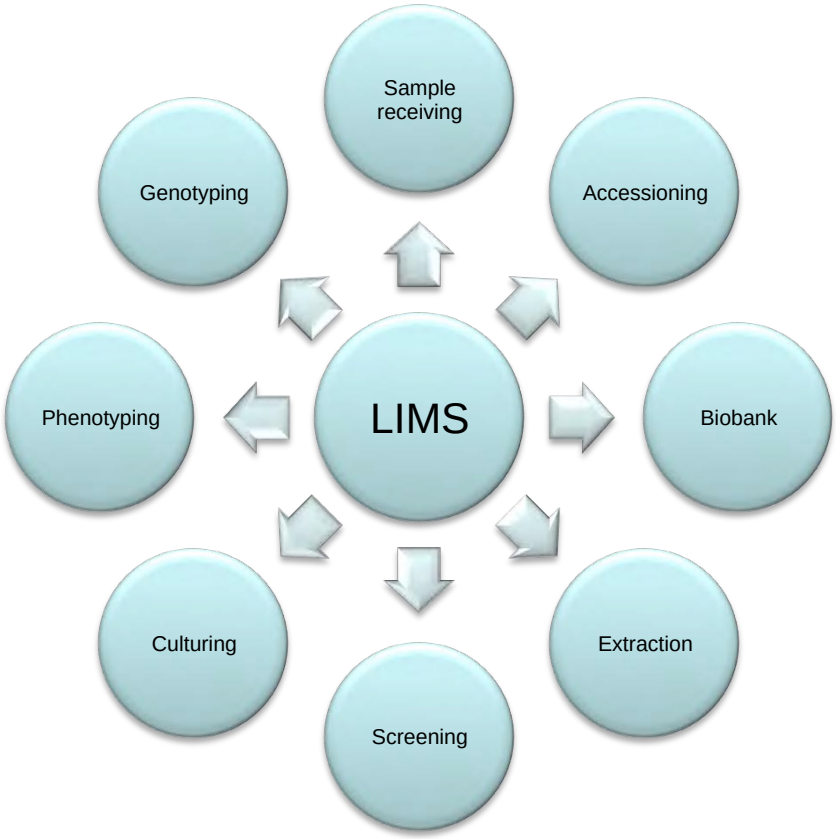


Figure 29. LIMS overview. All systems connect to the LIMS via a webservices interface.

Features

The design features for the HTLN LIMS are:

- Workflow management
  - Daily workorders will be generated and processed
  - Custom workorders may be necessary
  - "real-time" system status will be available (including current protocols in progress, protocol completion status, etc.)
- Sample/barcode verification against workflow
  - Each step of workflow will be checked against each sample ID to enforce workflow sequence and timing

    - All barcoded containers, including reagent plates, sample plates and sample tubes will be tracked and recorded
- Sample worklist generation (liquid handling transfer mapping from source to destination)
- Data acquisition (from data generating systems like plate readers, RT-PCR machines, etc.)
- Sample tracking
    - Each sample registered into HTLN will be assigned a unique ID
    - Each instance or aliquot of the sample will be tracked by container and platewell location
- Protocol management
    - The variable parameters for each protocol will be tracked and recorded
    - Protocol parameters will be "overideable" by lab manager/lab operators
    - Each protocol will be tracked for sequence and timing
    - Worklist management – custom cherry picking lists will be generated as needed
- Reports
    - Any current location of any sample should be queryable
    - The current status of any protocol should be queryable
    - A "dashboard" overview of laboratory operations

Webservice Interface

All communications (i.e., exchange of commands, data, and events) between the HTL OS and lab system will be implemented via Webservice-based interfaces (Figure 30). From a higher level, the communications between the HTL OS and a lab system will follow a traditional request/reply model, coupled with asynchronous event notification. A more detailed description of this Webservice-based interface can be found in the document "Software Requirements Specification: Software Control Interface for Laboratory Systems".

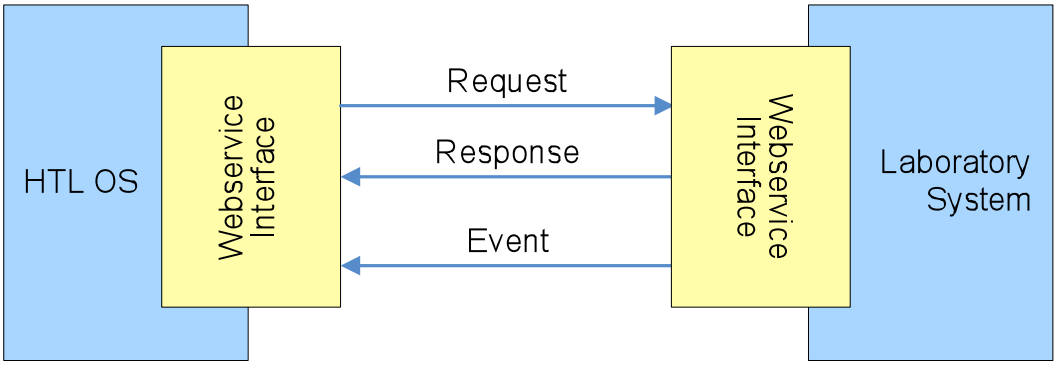


Figure 30: High-Level, Webservice-based System Interface

All information exchanged between the HTL OS and the system will be formatted in XML (eXtensible Markup Language). The necessary Webservice interface and XML schema definitions are provided in this document.

To effectively de-couple a lab system from the HTL LIMS (Laboratory Information Management System), the system will not have direct access to the LIMS. Instead, the HTL OS will be responsible for providing the system with all the required processing and sample information. Consequently, the HTL OS will be also in charge pulling processing-related data from the system (via a well-defined Webservice interface) and updating the LIMS accordingly.